

REMARKS

Status of the Claims

Claims 1-19 are pending.

Claims 20-21 are newly added.

Reconsideration is respectfully requested.

Amendments to the Specification

Applicants have amended the specification at page 11, line 1, to replace the incorrect reference to ring "B" with a correct reference to ring -- Z --. Support is found throughout the instant application.

Amendments to the Claims

The structure of formula (I) in Claim 1 was amended to remove the label "A" from inside the phenyl ring. Claim 1 was further amended, at line 3 thereof, to replace the word "its" with the expression -- or an --; to replace the plural words "enantiomers", "diastereomers", "salts" and "prodrugs" with the singular words -- enantiomer --, -- diastereomer --, -- salt -- and -- prodrug --, respectively; to replace the word "and" located before the word "pharmaceutically" with the word -- or --; to delete the comma after the word "salt"; to insert the word -- or - between the words "salt" and "prodrug"; and to delete the expression ", and" from the end of line 3. The word "solvates" was removed from line 4 of Claim 1. The definition of substituent R₆ in Claim 1 was amended to remove the label "A" from after the word "ring" and to insert the word -- the -- between the phrases "carbon atom of" and "phenyl ring."

Claim 2 was amended, at line 3 thereof, to insert the word -- or -- between the expression "salt," and the word "prodrug" and to delete the expression ", or solvate" therefrom. The structure of formula (I*) in claim 2 was amended to delete the label "A" from inside the phenyl ring.

Claims 3-15 were amended to delete the comma “,” after the word “salt”; to insert the word -- or -- between the words “salt” and “prodrug”; and to delete either of the expressions “or solvate” or “, or solvate” from between the words “prodrug” and “thereof.”

The definition of substituent R₆ in Claims 6 and 8 was amended to remove the label “A” from after the word “ring.” The definition of R₆ in Claim 6 was also amended to insert the word -- the -- between the phrases “carbon atom of” and “phenyl ring.” In Claim 8, the definition of substituent R₆ was further amended to insert the word -- the -- between the phrases “attached to” and “phenyl ring.”

The structure of the formula in Claim 16 was amended to remove the label “A” from inside the phenyl ring. Claim 16 was further amended, at line 3 thereof, to replace the word “its” with the expression -- or an --; to replace the plural words “enantiomers” and “diastereomers” with the singular words -- enantiomer -- and -- diastereomer --, respectively; to delete the comma after the word “salt”; to insert the word -- or - between the words “salt” and “prodrug”; and to remove the phrase “ or solvate” from after the word “prodrug.”

Claim 17 and 18 were amended to delete the phrase “at least” from between the words “comprising” and “one”; to insert the phrase -- or more -- after the word “one”; and to replace the singular word “compound” with the plural word -- compounds --.

Claim 20 was amended to add Alzheimer’s disease, multiple myeloma and myocardial ischemia to the list of preferred inflammatory disorders, the support for which is found on page 22 of the instant specification.

Multiple dependent Claim 21 is newly inserted to teach alkyl ester prodrugs of the compounds taught in Claims 1, 2 and 16. Support for the teachings of Claim 21 is found on pages 48-49 and 51-54 of the specification, where alkyl ester prodrugs are disclosed as Examples 48, 51, 52 and 56.

Applicants believe that the present amendments do not introduce new matter, are fully supported by the specification and priority document, and do not broaden the scope of the claims.

Rejections Under 35 U.S.C. §112, 2nd ¶

Claims 1-20 were rejected under the second paragraph of 35 U.S.C. §112 as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

1. The Examiner contends that recitation of “its enantiomers, diastereomers, pharmaceutically acceptable salts[,] prodrugs or solvates thereof” in Claim 1 is indefinite. Applicants have amended Claims 1 and 16 so that the above-cited recitation is in the singular form. Moreover, applicants have also amended Claim 1 to be in proper Markush alternate form.
2. The term “prodrug” was deemed indefinite. The Examiner points out that “the definition of various R[,] groups include such groups, namely esters, amides, alkoxycarbonyl etc. and therefore it is not clear what is the difference between these variable groups and the prodrug groups.” (Office Action of 03/21/2005, page 2.) Applicants believe the following explanation and the insertion of newly added Claim 21 will clarify this issue.

The present claims are written to cover both parent (active) compounds and prodrug compounds that mask the parent. The disclosure of the instant specification fully supports such claim language, as is evident from the disclosure of the alkyl ester prodrugs of Examples 48, 51, 52 and 56 on pages 48-49 and 51-54 of the specification and the disclosure of the parent compounds in the other Examples in the instant application. To further clarify the term “prodrug,” applicants have inserted Claim 21 to teach alkyl ester prodrugs. Moreover, while the “various R groups” do cover certain prodrugs, such as the alkyl ester prodrugs, claiming the term “prodrug” in the present case is intended to cover the prodrugs disclosed on pages 14-15 of the present specification.

The previous explanation should answer the Examiner’s contention that:

“if the variable groups are prodrug, which are in general inactive but becomes active upon in vivo transformation, then the compound bearing the variable group would be deemed as inactive which is not what the claims recite.”¹ (03/21/2005 O.A., p. 3.)

Applicants kindly reiterate that the present claims teach both parent and prodrug compounds. Thus, compounds bearing one variable group may be a parent compound, while compounds bearing

¹ Incidentally, applicants wish to point out that one of ordinary skill in the art would be of the opinion that not all prodrugs are inactive. See *infra*, pp. 14-15.

another variable group-- *e.g.*, the alkyl esters-- would be a prodrug. So, this should not render the instant claims indefinite.

In summary, applicants respectfully dispute the Examiner's assertion that:

“[a]pplicants' 'prodrugs' are molecules whose structure lie outside the subject matter of formula (I), but upon metabolism in the body are converted to active compounds falling within the structure scope of formula (I).” (03/21/2005 O.A., p. 3.)

As stated above, the relevant claims teach both prodrug and parent compounds, so applicants kindly submit that the Examiner's quoted assertion is not correct. Hence, applicants respectfully contend that the present claims clearly define the subject matter which applicants regard as the invention.

3. Claims 17-18 were alleged to be indefinite due to the recitation of the phrase “at least one of the compounds of.” Applicants have amended said claims in the manner suggested by the Examiner to replace the phrase “at least one of the compounds of” with the phrase -- one or more --.

4. The label “A” in the phenyl ring bearing R₆ was alleged to be confusing. As such, applicants have deleted label “A” from the definition of R₆ and from the chemical structures taught in Claims 1, 2, 6, 8 and 16.

For the above-stated reasons, applicants submit that the rejections under the second paragraph of 35 U.S.C. § 112 should be kindly withdrawn.

Rejections Under 35 U.S.C. §112, 1st ¶

Claims 1-20 were rejected under the first paragraph of 35 U.S.C. §112 because the specification supposedly “does not reasonably provide enablement for making prodrug[s] of the claimed compounds.” (03/21/2005 O.A., p. 4.) In discussing the first enablement factor under subsection a), the Examiner provides the following definition of a prodrug:

“It *must* itself be biologically inactive. It must be metabolized to a second substance at a physiologically meaningful concentration. Thirdly, that second substance must be biologically active.” (03/21/2005 O.A., p. 5 (emphasis added).)

Before responding to the substance of this rejection, applicants respectfully dispute the first prong of this 3-pronged definition of a prodrug. First, the Examiner does not cite a source for this definition. Second, one skilled in the art would disagree with the assertion that the prodrug “itself must be biologically inactive.” In fact, one reference defines an ideal ester prodrug as having “[w]eak (or no) activity against any pharmacological target,” among other desired properties. Kevin Beaumont, et al., *Design of Ester Prodrugs to Enhance Oral Absorption of Poorly Permeable Compounds: Challenges to the Discovery Scientist*, Curr. Drug Metabolism, 2003, 4, 461-486, 461 (attached herewith). Thus, contrary to the Examiner’s assertion, one skilled in the art would recognize that the prodrug itself can be biologically active.

As an aside, one skilled in the art would also recognize that a more accurate definition of a prodrug is summarized by the following quote:

“[t]he major aim of a prodrug approach is to mask polar or ionisable groups within a molecule. This increases the overall lipophilicity of the molecule and promotes membrane permeability and oral absorption.” *Id.* at 462.

Applicants respectfully further contend that the Examiner’s assertion in subsection c) of this non-enablement rejection-- *i.e.*, “[t]here is no working example of a prodrug of a compound [of] the formula (I)” (03/21/2005 O.A., p. 5.)-- is erroneous. As applicants point out hereinabove, pages 48-49 and 51-54 (Examples 48, 51, 52 and 56) of the instant specification clearly disclose alkyl ester prodrugs that are covered by the subject matter of Claims 1, 2 and 16, as well as the claims that depend therefrom. Applicants have also added depend Claim 21 to teach alkyl ester prodrugs specifically. Thus, applicants contend that this portion of the rejection is incorrect, as there are working examples of prodrugs in the present case.

Next, subsection e) of this rejection suggests that there is a low expectation of success, while subsection l) alleges that “a collaborative team of synthetic pharmaceutical chemists and metabolism experts” would be needed to make the prodrugs claimed herein, all of whom would have “a Ph.D. degree and several years of industrial experience.”² (03/21/2005 O.A., p. 5.) Applicants point out that *Beaumont, et al.* lists marketed ester prodrugs in Tables 1 to 6 therein, so it is not inconceivable for applicants to make such prodrugs successfully. As already discussed, applicants have, in fact,

made the alkyl ester prodrugs shown in Examples 48, 51, 52 and 56 of the instant application. Moreover, the inventors listed in the present case comprise mostly of Ph.D. chemists having numerous years of experience in the chemical and pharmaceutical field, such that their skill in the art would qualify them to make the prodrugs claimed herein.

As for subsection h) of this rejection, applicants kindly reiterate that they have made alkyl ester prodrugs to support the claim language for this case, so the breadth of the claims is reasonable.

In summary, for the above-stated reasons applicants believe that the teaching in the relevant claims of prodrugs is enabled by the disclosure of the instant specification, such that this rejection should be kindly removed.

Claims 1-20 were rejected under the first paragraph of 35 U.S.C. §112 for failing to meet the enablement requirement because the solvates of the claimed compounds are allegedly not enabled by the present specification. Applicants have removed the teaching of solvates from the instant claims, so this rejection is now obviated and should be kindly removed.

Claims 19 and 20 were rejected under the first paragraph of 35 U.S.C. §112 for allegedly “not reasonably provid[ing] enablement for treating any or all inflammatory disorder[s] embraced in the instant invention.” (03/21/2005 O.A., p. 8.) Applicants have included hereinbelow a summary of the inflammatory diseases implicated in the p38 pathway, for which there is ample evidence in the art showing that p38 inhibitors may be reasonably expected to treat. While this list is not exhaustive, it provides support for the subject matter taught in instant claims 19 and 20 and should obviate this non-enablement rejection.

Psoriasis

TNF α has been identified as a key mediator of the inflammation involved in psoriasis. The anti-TNF biologic etanercept has been approved for treatment of psoriasis. Approval by the FDA of etanercept for treatment of psoriasis demonstrates that this treatment is considered to be effective, thus providing a specific example that a drug that blocks TNF can successfully treat or slow the course of the disease. In a phase III clinical trial, etanercept gave a 75% improvement in the

² Subsection l) on page 5, of the March 21, 2005 Office Action should actually be subsection “f).”

Psoriasis Area and Severity Index (PASI) score in over half the patients at 24 weeks of treatment. Papp, K. A., et al., Brit. J. Dermatol., 152, 1304-1312 (2005) (a global phase III randomized controlled trial of etanercept in psoriasis: safety, efficacy, and effect of dose reduction). The ability of p38 inhibitors to block TNF is well established in the literature, *see* Kumar, S., et al., Nat. Rev. Drug Disc., 2, 717-726 (2003) (p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases); Saklatvala, J., Curr. Opin. Pharmacol., 4, 372-377 (2004) (p38 MAP kinase pathway as a therapeutic target in inflammatory disease), and has been demonstrated in clinical trials in human subjects. Branger, J., et al., J. Immunol., 168, 4070-4077 (2002) (anti-inflammatory effects of a p38 mitogen-activated protein kinase inhibitor during human endotoxemia). The p38 kinase has been found to be activated in psoriatic lesions. Johansen, C., et al., Brit. J. Dermatol., 152, 37-42 (2005) (the mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin). It is therefore reasonable to conclude that p38 inhibitors would have utility in the treatment of psoriasis.

Psoriatic Arthritis

Inhibition of TNF α has been demonstrated to be effective in the treatment of psoriatic arthritis, as exemplified by the FDA approval of the TNF blocking agents etanercept and infliximab for the treatment of psoriatic arthritis. Clinical trials of etanercept, infliximab and adalimumab (a fully human anti-TNF antibody) have demonstrated efficacy in psoriatic arthritis patients. Mease, P. J., and Antoni, C. E., Ann. Rheum. Dis., 64 Suppl 2, ii78-82 (2005) (psoriatic arthritis treatment: biological response modifiers). Etanercept demonstrated inhibition of disease progression, as measured by radiographic changes in clinical trials. Infliximab, in a 200 patient phase III study, demonstrated ACR20 scores in 58% of treated patients relative to 11% of placebo, and an average PASI score improvement in the ACR20 responders was 87%. As described above for psoriasis, p38 inhibitors are well established to be able to inhibit TNF production. Based on this evidence, as well as the evidence related to psoriasis and rheumatoid arthritis, p38 inhibitors are expected to have utility in the treatment of psoriatic arthritis.

Inflammatory Bowel Disease

Inhibition of TNF α has been demonstrated to be effective in the treatment of inflammatory bowel disease, as exemplified by the FDA approval of the TNF blocking agent infliximab for the

treatment of Crohn's Disease, a major type of inflammatory bowel disease. The use of infliximab in Crohn's disease has been well established. Sandborn, W.J. and Hanauer, S.B., Am. J. Gastroenterol., 2002, 97, 2962-2972 (infliximab in the treatment of Crohn's disease: a user's guide for clinicians); Sandborn, W.J. and Hanauer, S.B., Inflamm. Bowel Dis., 1999, 5, 119-133 (antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results, and safety). A clinical trial of a second monoclonal anti-TNF antibody, adalimumab, has also demonstrated efficacy. Youdim A., et al., Inflamm Bowel Dis., 2004, 10, 333-338 (a pilot study of adalimumab in infliximab-allergic patients). In addition, the blockade of TNF α by infliximab has utility in the treatment of ulcerative colitis, the second major type of inflammatory bowel disease, as demonstrated in clinical trials. Jarnerot, G., et al., Gastroenterology, 128, 1805-1811 (2005) (infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study); Travassos, W. J., and Cheifetz, A.S., Infliximab: Use in Inflammatory Bowel Disease, Curr. Treat. Options Gastroenterol., 8, 187-196 (2005). As described above for psoriasis, p38 inhibitors are well established to be able to inhibit TNF production. In addition, p38 kinase has been reported to be strongly activated in inflammatory bowel disease and linked to TNF α . Waetzig, G. H., et al., J. Immunol., 168, 5342-5351 (2002) (p38 mitogen-activated protein kinase is activated and linked to TNF α signaling in inflammatory bowel disease). Taken together, these findings support the expectation that p38 inhibitors would be of utility in the treatment of inflammatory bowel disease, including Crohn's disease and ulcerative colitis, through the mechanism of TNF blockade. An inhibitor of the p38 and Jnk kinases has been reported to be of benefit in a clinical trial of patients with severe Crohn's disease, further supporting the contention of utility of p38 inhibition. Hommes, D., et al., Gastroenterology, 122, 7-14 (2002) (inhibition of stress-activated MAP kinases induces clinical improvement in moderate to severe Crohn's disease). The expectation of utility has been published in the literature. Reimold, A. M., Curr. Drug Targets Inflamm. Allergy, 1, 377-392 (2002) (TNF α as therapeutic target: new drugs, more applications).

Ankylosing Spondylitis

Ankylosing spondylitis is a rheumatic disease of the spine, and therapies that have utility in the treatment of rheumatoid arthritis have utility in the treatment of this rheumatic disease as well. Specifically, etanercept has been approved by the FDA for treatment of ankylosing spondylitis, and

infliximab has been approved in the European Union for this indication. The utility of these anti-TNF antibodies has been demonstrated in clinical trials. Davis Jr, J. C., et al., Ann. Rheum. Dis. (2005) (sustained durability and tolerability of etanercept in ankylosing spondylitis for 96 weeks); Davis, J. C., Jr., Semin. Arthritis Rheum., 34, 668-677 (2005) (understanding the role of tumor necrosis factor inhibition in ankylosing spondylitis). These findings demonstrate the utility of TNF blockade in the treatment of ankylosing spondylitis. Since p38 inhibitors block TNF production, p38 inhibitors are expected to have utility in the treatment of this disease. In addition, p38 kinase has been reported to be expressed at elevated levels in circulating monocytes of ankylosing spondylitis patients relative to normal controls, providing further support for the utility of inhibiting p38 kinase in the treatment of this disease. Gu, J., et al., Rheumatology, 41, 759-766 (Oxford 2002) (a 588-gene micro-array analysis of the peripheral blood mononuclear cells of spondyloarthropathy patients).

Osteoarthritis

p38 inhibitors are expected to have utility in the treatment of osteoarthritis due to their ability to relieve pain. In addition, p38 inhibitors offer the opportunity to act as disease modifying osteoarthritis drugs due to inhibition of production of IL-1 β , MMP-13, and other potential mediators of joint destruction in osteoarthritis. This additional activity provides further support for the utility in the treatment of osteoarthritis. Numerous studies have reported data supporting the utility of p38 kinase inhibition in the treatment of pain, due to inhibition of expression of COX-2 and other mediators likely to contribute to pain. In addition to its role in pain responses generated by peripheral expression of COX-2, p38 kinase is believed to play an important role in the central nervous system through its action in glial cells in pain responses. Inhibition of COX-2 expression and production of pro-inflammatory cytokines, such as TNF α , by glial cells has been proposed to offer new opportunities for treatment of pain facilitation, in which stimuli are perceived as being either more painful than they would normally be (hyperalgesia) or as painful when normally they would not be painful (allodynia). Watkins, L. R., et al., Adv. Exp. Med. Biol., 521, 1-21 (2003) (glial pro-inflammatory cytokines mediate exaggerated pain states: implications for clinical pain). For example, in thermal hyperalgesia, warmth is perceived as pain, and in mechanical allodynia, touch is perceived as pain. Such pain facilitation can result in perception of pain after the initiating injury has resolved, leading to chronic pain that is poorly controlled by current drug therapy.

Activation of p38 by nerve growth factor (NGF) in primary sensory neurons after inflammation increases levels of the transient receptor potential ion channel TRPV1, also known as vanilloid receptor-1 (VR-1). Ji, R.R., et al., Neuron, 36, 57-68 (2002) (p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia). The p38 mediated expression of this heat gated ion channel contributes to the maintenance of inflammatory heat hyperalgesia. Direct administration of SB-203580 to the central nervous system by intrathecal infusion prevented inflammatory heat hyperalgesia in an animal model. Intrathecal infusion of SB-203580 has also been reported to be efficacious in treatment of mechanical allodynia in surgical models of neuropathy. Jin, S. X., et al., J. Neurosci., 23, 4017-4022 (2003) (p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain); Schafers, M., et al., Neurosci., 23, 2517-2521 (2003) (tumor necrosis factor-alpha induces mechanical allodynia after spinal nerve ligation by activation of p38 MAPK in primary sensory neurons). Intrathecal administration of the Scios p38 inhibitor SD-282 has been reported to be effective in all models of NMDA-induced prostaglandin E2 release in the spine, as well as in models of thermal hyperalgesia, direct spinal activation by intrathecal substance P, and peripheral tissue inflammation, such as intraplantar formalin and carrageenan hyperalgesia models. Svensson, C. I., et al., Neuroreport, 14, 1153-1157 (2003a) (spinal p38 MAP kinase is necessary for NMDA-induced spinal PGE(2) release and thermal hyperalgesia); Svensson, C. I., et al., J. Neurochem., 86, 1534-1544 (2003b) (activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing). Importantly, clinical data demonstrates that oral administration of SCIO-469 was efficacious in a PhIIa dental pain study. Tong, S., et al., Clin. Pharmacol. Ther., 75, P3 (2004) (SCIO-469, a novel P38A MAPK Inhibitor, provides efficacy in acute post-surgical dental pain).

Osteoporosis

p38 kinase plays a role in the differentiation of osteoclasts induced by RANKL. Matsumoto, M., et al., J. Biol. Chem., 275, 31155-31161 (2000) (involvement of p38 mitogen-activated protein kinase signaling pathway in osteoclastogenesis mediated by receptor activator of NF-kappa B ligand (RANKL)); Wei, S., et al., J. Clin. Invest., 115, 282-290 (2005) (IL-1 mediates TNF-induced

osteoclastogenesis). RANKL is an essential cytokine for the formation and activation of osteoclasts, and activated T cells are an important source of RANKL in rheumatoid arthritis. Hofbauer, L. C., and Schoppet, M., JAMA, 292, 490-495 (2004) (clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases). RANKL is also believed to play a key role in the bone resorption in psoriatic arthritis. Ritchlin, C. T., et al., J. Clin. Invest., 111, 821-831 (2003) (mechanisms of TNF-alpha- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis). Since osteoclasts are the cells that are directly implicated in the formation of joint erosions, this provides an important mechanism by which p38 inhibition is expected to protect against bone destruction. A substantial proportion of RA and ankylosing spondylitis patients are either at risk for, or have developed, osteoporosis. Jolles, B. M., and Bogoch, E. R., J. Rheum., 29, 1814-1817 (2002) (current consensus recommendations for rheumatoid arthritis therapy: a blind spot for osteoporosis prevention and treatment); Gough, A., et al., Lancet, 344, 23-27 (1994) (generalized bone loss in patients with early rheumatoid arthritis). Osteoclast activity is believed to be the principal mechanism by which osteoporosis occurs in RA patients. Gough, A., et al., J. Rheum., 25, 1282-1289 (1998) (osteoclastic activation is the principal mechanism leading to secondary osteoporosis in rheumatoid arthritis).

AMG-162 is a monoclonal antibody from Amgen that binds RANKL, blocking binding of RANKL to its receptor, RANK. In a single dose study in osteoporosis patients, AMG-162 provided a dose-dependent rapid and sustained decrease from baseline in bone turnover as measured by multiple biomarkers, suggesting its potential in the treatment of osteoporosis. Bekker, P. J., et al., J. Bone Miner. Res., 19, 1059-1066 (2004) (a single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women). Since p38 inhibitors can block osteoclast formation through their action on RANK signal transduction, (Matsumoto et al., 2000), p38 inhibitors are expected to have utility in the treatment of osteoporosis.

Atherosclerosis

There is compelling evidence for the role of inflammation, particularly as indicated by elevated CRP levels, in coronary artery disease. Nissen, S. E., et al., N. Engl. J. Med., 352, 29-38 (2005) (statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease); Ridker, P.M., et al., N. Engl. J. Med., 352, 20-28 (2005) (C-reactive protein levels and outcomes after statin

therapy). p38 inhibitors have the potential to be of value in the treatment of atherosclerosis due to their anti-inflammatory activity. In particular, p38 inhibitors affect multiple mediators important in atherosclerotic plaque formation and destabilization, such as CRP, TNF, IL-1, MCP-1, and MMP-13. In addition, p38 activity has been reported to be necessary for foam cell formation, Zhao, M., et al., Apmis, 110, 458-468 (2002) (activation of the p38 MAP kinase pathway is required for foam cell formation from macrophages exposed to oxidized LDL), and thrombin induced vascular smooth muscle migration, Wang, Z., et al., J. Mol. Cell. Cardiol., 36, 49-56 (2004) (reactive oxygen species-sensitive p38 MAPK controls thrombin-induced migration of vascular smooth muscle cells), offering additional mechanisms for potential benefit in atherosclerosis. Heterozygous p38 α +/- mice displayed increased times to thrombotic occlusion compared with wild type mice in the ferric chloride model of thrombus formation, Wang, et al. (2004), whereas a selective COX-2 inhibitor decreased the time to occlusion in a similar ferric chloride model. Buerkle, M.A., et al., Circulation, 110, 2053-2059 (2004) (selective inhibition of cyclooxygenase-2 enhances platelet adhesion in hamster arterioles in vivo). The p38 α +/- mice displayed lower expression and activity of tissue factor in the model. The p38 inhibitor BIRB-796 was reported to strongly inhibit LPS-induced coagulation activation in human subjects, as measured by plasma concentrations of the prothrombin fragment F1 + 2. Branger, J., et al., Blood, 101, 4446-4448 (2003) (inhibition of coagulation, fibrinolysis, and endothelial cell activation by a p38 mitogen-activated protein kinase inhibitor during human endotoxemia). In addition, the p38 α +/- mice displayed impaired aggregation and binding to fibrinogen following stimulation with a thromboxane A2 analog. Wang, et al. (2004). Multiple studies indicate that p38 inhibitors would be of value in the treatment of ischemia and reperfusion injury. VX-702 has been tested in a PhII study of acute coronary syndrome, and CRP levels were reported to be markedly reduced in treated patients relative to placebo controls. Taken together, these reports support the utility of p38 inhibition in the treatment of atherosclerosis and related cardiovascular disease.

Ischemia/Reperfusion Injury – Acute Coronary Syndrome and Stroke

Multiple reports support the utility of p38 inhibition in ischemia reperfusion injury, which occurs in myocardial infarction and stroke. Mice expressing a dominant negative p38 kinase mutant showed marked protection from persistent cardiomyocyte apoptosis in a cardiac ischemia model. Ren, J., et al., J. Mol. Cell. Cardiol., 38, 617-623 (2005) (role of p38 α MAPK in cardiac

apoptosis and remodeling after myocardial infarction). Inhibition of p38 kinase protects the heart against cardiac remodeling in mice with heart failure resulting from myocardial infarction. Li, Z., et al., J. Cardiovasc. Pharmacol., 44, 486-492 (2004) (p38 alpha mitogen-activated protein kinase inhibition improves cardiac function and reduces myocardial damage in isoproterenol-induced acute myocardial injury in rats); Liu, Y.H., et al., J. Cardio. Fail., 11, 74-81 (2005) (inhibition of p38 mitogen-activated protein kinase protects the heart against cardiac remodeling in mice with heart failure resulting from myocardial infarction); See, F., et al., J. Am. Coll. Cardiol., 44, 1679-1689 (2004) (p38 mitogen-activated protein kinase inhibition improves cardiac function and attenuates left ventricular remodeling following myocardial infarction in the rat). p38 can form a stable complex with MAPKAP kinases, and phosphorylation of these downstream kinases can unmask a nuclear localization signal that permits the translocation of the MAPKAP-p38 complex to the nucleus. MAPKAP K2, also known as MK2, is believed to be among the most important p38 substrates in mediating pro-inflammatory effects based on the phenotype of MAPKAP K2 knockout mice. Kotlyarov, A., et al., Nat. Cell. Biol., 1, 94-97 (1999) (MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis). However, since MAPKAP K2 serves to stabilize p38 α , MAPKAP K2 knockout mice also have significantly reduced expression of p38 kinase. Kotlyarov, A., et al., Mol. Cell. Biol., 22, 4827-4835 (2002) (distinct cellular functions of MK2). Whether the reduced inflammatory responses in MAPKAP K2 knockout mice are due directly to lack of MAPKAP K2 or are due at least in part indirectly to lack of p38 kinase, they provide additional validation for p38 as a target. MAPKAP K2 knockout mice were resistant to myocardial ischemic reperfusion injury as evidenced by enhanced recovery of post-ischemic ventricular performance, reduced myocardial infarct size and diminished number of apoptotic cardiomyocytes. Shiroto, K., et al., J. Mol. Cell. Cardiol., 38, 93-97 (2005) (MK2-/- gene knockout mouse hearts carry anti-apoptotic signal and are resistant to ischemia reperfusion injury). In addition, the brains of MAPKAP K2 knockout mice are protected from ischemic injury compared to wild-type mice, displaying greatly reduced infarct size and significant reduction in neurological deficits. Wang, X., et al., J. Biol. Chem., 277, 43968-43972 (2002) (mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 deficiency protects brain from ischemic injury in mice). Taken together, this genetic and pharmacologic evidence supports the utility of p38 inhibition in the treatment of ischemia.

COPD

Inhibitors of p38 have been reported to be efficacious in animal models of pulmonary inflammation. Merck and Glaxo p38 inhibitors have been reported to show efficacy in preclinical LPS lung acute inflammation models, Haddad, E.B., et al., *Brit. J. Pharmacol.*, *132*, 1715-1724 (2001) (role of p38 MAP kinase in LPS-induced airway inflammation in the rat); Nick, J.A., et al., *J. Immunol.*, *169*, 5260-5269 (2002) (selective suppression of neutrophil accumulation in ongoing pulmonary inflammation by systemic inhibition of p38 mitogen-activated protein kinase); Nick, J.A., et al., *J. Immunol.*, *164*, 2151-2159 (2000) (role of p38 mitogen-activated protein kinase in a murine model of pulmonary inflammation), while Glaxo has disclosed efficacy in preclinical bleomycin lung inflammation models. Underwood, D.C., et al., *Am. J. Physiol. Lung Cell. Mol. Physiol.*, *279*, L895-902 (2000a) (SB 239063, a p38 MAPK inhibitor, reduces neutrophilia, inflammatory cytokines, MMP-9, and fibrosis in lung). This and other data has led to recognition in the scientific literature of the utility of p38 kinase inhibition for treatment of COPD. Adcock, I.M., and Caramori, G., *BioDrugs*, *18*, 167-180 (2004) (kinase targets and inhibitors for the treatment of airway inflammatory diseases: the next generation of drugs for severe asthma and COPD); Barnes, P.J., and Hansel, T.T., *Lancet*, *364*, 985-996 (2004) (prospects for new drugs for chronic obstructive pulmonary disease); Donnelly, L.E., and Rogers, D.F., *Drugs*, *63*, 1973-1998 (2003) (therapy for chronic obstructive pulmonary disease in the 21st century).

Multiple Myeloma

Multiple myeloma is a malignancy of plasma B cells which induce osteolytic bone lesions. A variety of autocrine and paracrine factors, including IL-6 and VEGF, are believed to be important in the growth of multiple myeloma cells, which interact with bone marrow stromal cells to induce production of these factors. Hideshima, T., et al., *Blood*, *101*, 703-705 (2003) (targeting p38 MAPK inhibits multiple myeloma cell growth in the bone marrow milieu); Plataniias, L.C., *Blood*, *101*, 4667-4679 (2003) (map kinase signaling pathways and hematologic malignancies). The Vertex p38 inhibitor, VX-745, has been reported to inhibit IL-6 and VEGF secretion in bone marrow stromal cells and inhibit paracrine induced multiple myeloma cell growth. *Hideshima, et al.*(2003). Multiple myeloma cell induced production of RANKL and MIP1 α is believed to be important in the formation and activation of osteoclasts that lead to the osteolytic bone lesions characteristic of this

disease. Giuliani, N., et al., *Exp. Hematol.*, 32, 685-691 (2004) (new insight in the mechanism of osteoclast activation and formation in multiple myeloma: focus on the receptor activator of NF-kappaB ligand (RANKL)). Inhibition of p38 kinase may potentially inhibit osteolytic lesions, since p38 has been reported to regulate production of both RANKL and RANKL-induced osteoclast differentiation. Li, X., et al., *Endocrinology*, 143, 3105-3113 (2002) (p38 MAPK-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function); Matsumoto, et al. (2000); Wang, S.W., et al., *Inflamm. Res.*, 48, 533-538 (1999) (cytokine mRNA decay is accelerated by an inhibitor of p38-mitogen-activated protein kinase); Wei, et al., 2005. Production of MIP1 α has been reported to be regulated by p38 inhibitors through control of message stability. Matsuyama, W., et al., *J. Immunol.*, 172, 2332-2340 (2004) (activation of discoidin domain receptor 1 isoform b with collagen up-regulates chemokine production in human macrophages: role of p38 mitogen-activated protein kinase and NF-kappa B); Wang, et al. (1999). In addition, the Scios p38 inhibitor, SCIO-469, has been reported to enhance the susceptibility of multiple myeloma cells to killing by proteasome inhibitors. Hideshima, T., et al., *Oncogene*, 23, 8766-8776 (2004) (p38 MAPK inhibition enhances PS-341 (bortezomib)-induced cytotoxicity against multiple myeloma cells). Thus, p38 inhibition may be expected to be of benefit in the treatment of multiple myeloma by inhibiting tumor cell growth, sensitizing tumor cells to chemotherapy, and inhibiting the osteolytic bone lesions that are characteristic of the disease.

Gouty arthritis

The formation and deposition of monosodium urate (MSU) microcrystals in articular and periarticular tissues is the causative agent of acute or chronic inflammatory responses known as gouty arthritis. Monocyte activation is involved in early triggering events of gout attacks. COX-2 expression and PGE(2) production is an important mechanism in this process. MSU crystals have been found to induce COX-2 in human monocytes, and p38 inhibition was found to block this effect. Pouliot, M., et al., *Blood*, 91, 1769-1776 (1998) (monosodium urate microcrystals induce cyclooxygenase-2 in human monocytes). p38 kinase inhibitors are, therefore, expected to have utility in the treatment of gouty arthritis by inhibiting the monocyte responses to MSU crystals, in particular the expression of COX-2.

Asthma

Multiple p38 kinase inhibitors have been reported to be effective in animal models of asthma. Escott, K.J., et al., Br. J. Pharmacol., 131, 173-176 (2000) (effect of the p38 kinase inhibitor, SB 203580, on allergic airway inflammation in the rat); Underwood, D.C., et al., J. Pharmacol. Exp. Ther., 293, 281-288 (2000b) (SB 239063, a potent p38 MAP kinase inhibitor, reduces inflammatory cytokine production, airways eosinophil infiltration and persistence). In addition to this evidence from pharmacologic inhibition of p38 kinase activity, antisense oligonucleotides that block the expression of p38 kinase protein have also been reported to be effective in an animal model of asthma. Duan, W., et al., Am. J. Respir. Crit. Care Med., 171, 571-578 (2005) (inhaled p38alpha mitogen-activated protein kinase antisense oligonucleotide attenuates asthma in mice). This and other data has led to recognition in the scientific literature of the utility of p38 kinase inhibitors in the treatment of asthma. Barnes, P. J., Nat. Rev. Drug Discov., 3, 831-844 (2004) (new drugs for asthma); Newton, R., and Holden, N., BioDrugs, 17, 113-129 (2003) (inhibitors of p38 mitogen-activated protein kinase: potential as anti-inflammatory agents in asthma).

Diabetes

There are multiple lines of evidence that p38 inhibitors would be useful in the treatment of diabetes. High glucose levels associated with diabetes have been shown in animal models to induce activation of p38 kinase in tissues affected by the disease. Specifically, in the streptozotocin (STZ)-induced diabetes model in the rat, activation of p38 kinase was observed in the thoracic aorta and kidney cortex, in conjunction with renal and vascular dysfunction. Chen, H., et al., "Duration of streptozotocin-induced diabetes differentially affects p38-mitogen-activated protein kinase (MAPK) phosphorylation in renal and vascular dysfunction," Cardiovasc. Diabetol., 4(1), 3 (2005). In addition, elevated levels of activated p38 have been observed in human subjects with diabetes. Adhikary, L., et al., "Abnormal p38 mitogen-activated protein kinase signaling in human and experimental diabetic nephropathy," Diabetologia, 47(7), 1210-22 (2004). In non-obese diabetic (NOD) mice, phosphorylated p38 MAPK was observed immunohistochemically in CD4+ cells that had infiltrated into the islets and part of beta-cells, increasing in proportion to the severity of insulinitis. Treatment of the mice with a p38 inhibitor prevented the NOD mice from developing diabetes without affecting the severity of insulinitis. Treatment with the inhibitor after insulinitis had developed also prevented diabetes, further suggesting that treatment with the p38 MAPK pathway inhibitor keeps insulinitis benign in NOD mice. Ando, H., et al., "The specific p38 mitogen-activated

protein kinase pathway inhibitor FR167653 keeps insulinitis benign in nonobese diabetic mice," Life Sci., 74(14), 1817-27 (2004). Similar results have been reported for other p38 inhibitors, including improvement in hypoglycemia. In NOD mice treated for 10 weeks with standard chow or chow containing high and low doses of the p38 inhibitor SD-169, SD-169 treatment prevented the incidence of diabetes and alleviated hypoglycemia in a dose dependent manner. SD-169 also reversed p38 expression in T cells from the pancreatic islets. Using mildly hyperglycemic mice, SD-169 improved glucose homeostasis. Medicherla, S., et al., "p38 MAPK Inhibitor Prevents the Development of Type-1 Diabetes and Alleviates Hyperglycemia in NOD Mice: A Preliminary Report," Diabetes, 52(Suppl 6), Abs 542-P (2003). In a related study with a different p38 inhibitor, 8-week old NOD mice were treated for 10 weeks with standard chow with vehicle or chow containing the p38 kinase inhibitor SX-004. SX-004 treatment prevented the incidence of diabetes, as determined by blood glucose levels, by 18 weeks. Immunohistochemistry of pancreatic beta islet tissue demonstrated significant reduction in CD5+ T-cell infiltration. Medicherla, S., et al., "Further Studies on p38alpha Specific MAPK Inhibitor Prevents the Development of Type-1 Diabetes and Alleviates Hyperglycemia in NOD Mice," Diabetes, 54(6 Suppl 1), Abs 268-OR (2005). Taken together, these reports of increased p38 activity in human diabetes patients and animal models, plus the prevention of onset of diabetes and improvement in hyperglycemia in preclinical models, support the utility of p38 inhibitors in the treatment of diabetes.

In summary, given the disclosure in the application and the state of the art of immunology, Applicants assert that the claim to treating inflammation and/or inflammatory diseases is fully enabled. It is requested that the Examiner withdraw these rejections under 35 U.S.C. § 112, first paragraph.

Fees

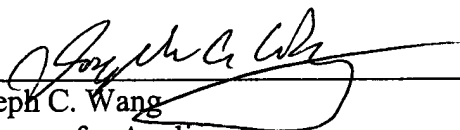
If it is determined that a fee is due, please charge same to Deposit Account No. 19-3880 in the name of Bristol-Myers Squibb Company.

SUMMARY

In view of the foregoing comments and amendments, applicants kindly request reconsideration of the application. Applicants believe the case is now in condition for allowance and respectfully request the Examiner to pass the case to issue at an early date.

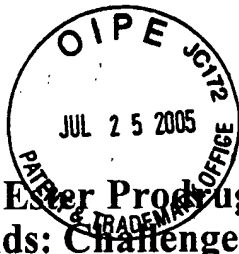
Respectfully submitted,

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Joseph C. Wang
Attorney for Applicants
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Date: July st 21 2005



Design of Ester Prodrugs to Enhance Oral Absorption of Poorly Permeable Compounds: Challenges to the Discovery Scientist

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Abstract: Many drugs are administered at sites that are remote from their site of action. The most common route of drug delivery is the oral route. The optimal physicochemical properties to allow high transcellular absorption following oral administration are well established and include a limit on molecular size, hydrogen bonding potential and adequate lipophilicity.

For many drug targets, synthetic strategies can be devised to balance the physicochemical properties required for high transcellular absorption and the SAR for the drug target. However, there are drug targets where the SAR requires properties at odds with good membrane permeability. These include a requirement for significant polarity and groups that exhibit high hydrogen bonding potential such as carboxylic acids and alcohols. In such cases, prodrug strategies have been employed.

The rationale behind the prodrug strategy is to introduce lipophilicity and mask hydrogen bonding groups of an active compound by the addition of another moiety, most commonly an ester. An ideal ester prodrug should exhibit the following properties:

- 1) Weak (or no) activity against any pharmacological target,
- 2) Chemical stability across a pH range,
- 3) High aqueous solubility,
- 4) Good transcellular absorption,
- 5) Resistance to hydrolysis during the absorption phase,
- 6) Rapid and quantitative breakdown to yield high circulating concentrations of the active component post absorption.

This paper will review the literature around marketed prodrugs and determine the most appropriate prodrug characteristics. In addition, it will examine potential Discovery approaches to optimising prodrug delivery and recommend a strategy for prosecuting an oral prodrug approach.

INTRODUCTION

For patient convenience, most drugs are administered by the oral route. However, there are significant hurdles confronting the delivery of a drug from the oral route, which often means that all of the administered compound does not reach its intended site of action. The extent to which the compound can overcome the hurdles to oral drug delivery and reach the systemic circulation is quantified by the term oral bioavailability. Optimising the oral bioavailability of a candidate molecule is a key objective of an oral drug discovery program. Clearly, compounds exhibiting low oral bioavailability are likely to require high doses to achieve the desired effects, since systemic exposure to the active compound will be limited. In addition, low oral bioavailability agents are prone to exhibit greater variation in exposure than higher bioavailability agents. This is particularly an issue in

the area of drug-drug interactions where concomitantly administered drugs can lead to unacceptable systemic exposure to a drug.

The factors limiting the oral bioavailability of drug molecules are well established. They fall into 2 broad categories: absorption from the g.i. tract and first-pass extraction by the gut wall and liver. Incomplete oral absorption can be a major cause of poor oral bioavailability and much research has focused on the physicochemical properties required to ensure high oral absorption.

The major physicochemical determinants of extensive passive transcellular oral absorption have been extensively studied. The Lipinski 'rule of 5' mnemonic [1] suggests that in order to exhibit good oral absorption in humans, a drug should possess a molecular weight lower than 500, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and a clog P less than 5. In addition, it is well known that in order to be transcellularly absorbed, a drug must possess a degree of lipophilicity (in general log $D_{(7.4)}$ greater than 0). These physicochemical constraints are related to the requirement of the drug to pass through the lipophilic environment of the gut wall cell membrane.

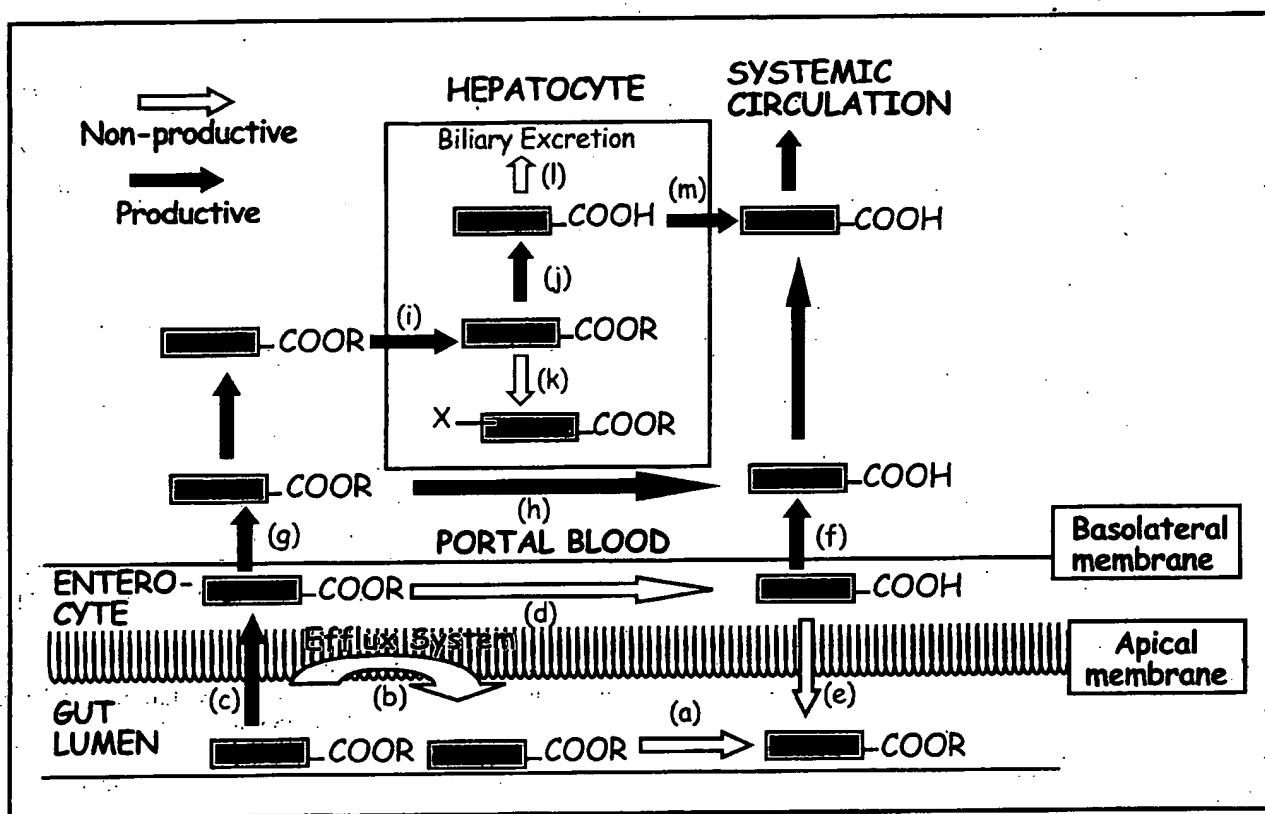
*Address correspondence to this author at the Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent, UK. CT13 9NJ, USA; Tel: (01304) 616161; Fax: (01304) 651817; E-mail: kevin_beaumont@sandwich.pfizer.com

However, there are pharmacological targets (eg GpIIb/IIIa antagonists, ACE inhibitors, viral reverse transcriptase inhibitors etc.) for which some chemical series require physicochemical properties that are at odds with good transcellular permeation. Thus, drug intervention at these targets can require compounds that are highly polar, have high molecular weight and/or high hydrogen bonding functionality. In order to make such compounds amenable to oral delivery, it is necessary to temporarily address these physicochemical constraints in a manner that can be readily reversed post absorption. This is the rationale behind the prodrug approach.

The major aim of a prodrug approach is to mask polar or ionisable groups within a molecule. This increases the overall lipophilicity of the molecule and promotes membrane permeability and oral absorption. However, increases in lipophilicity produced by a prodrug approach do not inevitably lead to major improvements in oral bioavailability. This

is due to the multiplicity of the barriers facing oral delivery. These are outlined in Fig. (1).

In order to be absorbed, the prodrug must be in solution in the contents of the gut. Thus, it must exhibit sufficient aqueous solubility to dissolve the entire dose. Once in solution, the prodrug needs to avoid extensive chemical and enzymic degradation, and must pass through the membrane of the gut wall cells (enterocyte). It is well known that efflux transporters are present in the enterocyte membrane [2], which are capable of intercepting drugs during membrane passage and placing them back into the gut lumen. Thus, successful prodrugs may need to avoid affinity for these transport proteins. Enterocytes are metabolically competent cells that express a wide range of drug metabolising enzymes including esterases [3], cytochrome P450 isoforms [3-5] and UDP-glucuronyl transferases [3,6]. Prodrug ester hydrolysis in the enterocyte can be productive, if the active principle



- (a) Chemical instability or degradation by gut lumen esterases,
- (b) Efflux of ester from the enterocyte membrane by a transporter mechanism (such as P-glycoprotein),
- (c) Permeation of ester through the apical membrane of the enterocyte,
- (d) Breakdown of the ester to the acid by enterocyte esterases,
- (e) Return of acid to gut lumen (possibly by an efflux system),
- (f) Crossing of the basolateral membrane of the enterocyte by the acid,
- (g) Crossing of the basolateral membrane of the enterocyte by the ester,
- (h) Hydrolysis of the ester by esterases in the portal vein blood,
- (i) Hepatic extraction of the ester,
- (j) Hydrolysis of the ester by hepatocyte esterases,
- (k) Non-esterase metabolism of the ester (such as CYP-mediated metabolism),
- (l) Biliary excretion of the acid,
- (m) Return of the liberated acid to the systemic circulation (probably transporter mediated).

Fig. (1). Barriers confronting the oral delivery of a prodrug.

can pass through the basolateral membrane of the enterocyte and into the blood. However, if the active principle is a substrate for apical membrane transporters, it is most likely to be returned to the gut lumen. Any cytochrome P450 or conjugation metabolism on passage through the enterocyte will also be observed as incomplete oral bioavailability of the active principle.

Post absorption, the ideal prodrug should rapidly and quantitatively be converted to the active principle. Our analysis suggests that this is rarely the case, due to the multiplicity of enzyme systems available to metabolise drugs on first-pass through the liver. Esterase enzymes present in the blood are capable of hydrolysing ester prodrugs. However, these esterase enzymes have a strict structure activity relationship and do not metabolise all esters. Therefore some ester prodrugs do require hepatic ester hydrolysis and this poses several issues for quantitative release of active principle. Following hepatic ester hydrolysis, the active principle will be released in the hepatocyte. Since the aim of a prodrug strategy is to improve membrane permeation, there is potential for the active principle to be trapped within the hepatocyte and require active efflux from the cell. Should the active principle be a preferential substrate for canalicular transporters, it will be extensively excreted into the bile. In order to be returned to the blood, it may require efflux by sinusoidal transporters. In addition, the hepatocyte also expresses high levels of Phase I and II metabolising enzymes that may metabolise the prodrug or the active principle in a non-productive manner. Thus, any metabolism or excretion of either the prodrug or the active principle, which does not lead to the transfer of the active principle to the blood, will be non-productive and lead to a lowering of potential oral bioavailability.

Overall, the barriers confronting the oral delivery of prodrugs are considerable. In addition, to improving membrane permeability of a polar active principle, a prodrug should avoid transporter mediated efflux and be designed to yield the active principle into the systemic circulation. Incomplete membrane permeation, efflux, non-esterase metabolism or biliary elimination will lead to a reduction in the potential oral bioavailability of the active principle. Thus, in order to be successful, a prodrug approach must consider the balance of all these issues.

This paper examines many literature examples illustrating the issues facing the prodrug approach. It will attempt to review the oral bioavailability of marketed prodrugs and outline examples of prodrug approaches that have failed. Finally, an attempt will be made to outline a strategy for the Discovery scientist to successfully prosecute a prodrug approach.

REVIEW OF MARKETED ESTER PRODRUGS

A number of examples of marketed ester prodrugs are listed in Tables 1 to 6. The prodrugs show a wide diversity in chemical structure, molecular weight and lipophilicity. However, relatively few different classes of ester groups have been employed as the prodrug moiety. In general, the highest oral bioavailability values that can be achieved clinically using ester prodrugs are 40-60%, indicating that complete oral bioavailability in a prodrug programme is very unlikely.

ALKYL ESTERS

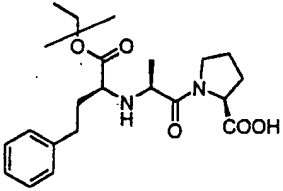
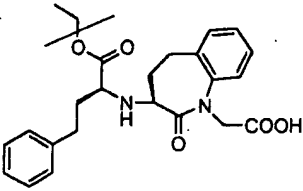
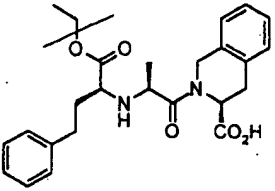
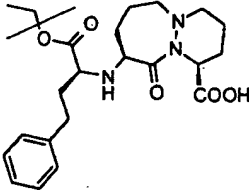
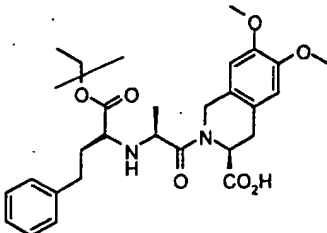
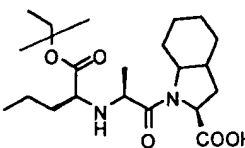
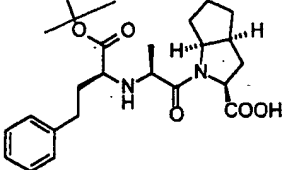
The most common prodrug moiety in marketed drugs is the esterification of an acid group with a simple alkyl alcohol. Major examples are illustrated in Table 1. One of the most successful classes of alkyl ester prodrugs is the diacidic angiotensin converting enzyme (ACE) inhibitors, where one of the acidic functions is masked as an ethyl ester. The oral bioavailability values for the diacidic active principles (eg benazeprilat, quinaprilat etc) are not widely reported but are likely to be low. For instance, the oral absorption of enalaprilat is only 3% [7] and the bioavailability of cilazaprilat is 19% [8]. In contrast, the oral bioavailability values of most of the monoacid prodrug agents (enalapril, benazepril, quinapril etc.) are significantly higher than 20%. However, even within this class the oral bioavailability values exhibit a significant range, from 17.5 % for moexipril to 57% for cilazapril.

There are a number of reasons why the monoacid ester pro-drugs have higher bioavailability than their respective diacid active principle and why there is a large variability in bioavailability between different ACE inhibitors. Firstly, changing one of the acid groups to an ethyl ester increases lipophilicity with a resultant increase in transcellular absorption. However, a number of the monoester ACE inhibitors exhibit greater oral absorption than would be expected purely from their lipophilicity values. For example, enalapril is relatively hydrophilic but is well absorbed in humans. It appears that as well as increasing lipophilicity, the monocarboxylic acid ester ACE inhibitors are substrates for intestinal uptake transporters [9-15]. In contrast although a number of di-acid ACE inhibitors can interact with the intestinal uptake transporters the second negative charge of these compounds makes their transport by these proteins unfavourable [12,16], and this in part explains their lower bioavailability.

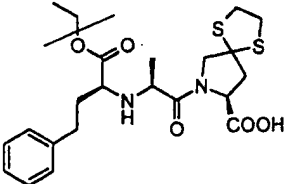
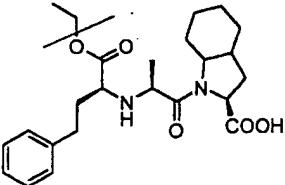
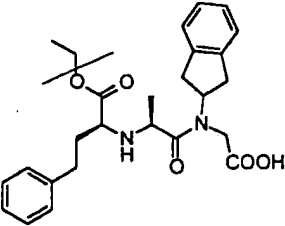
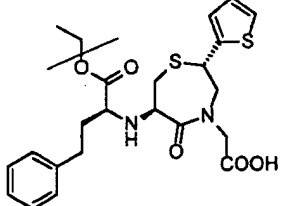
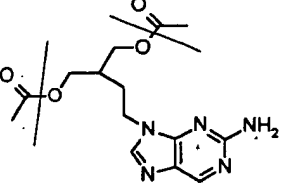
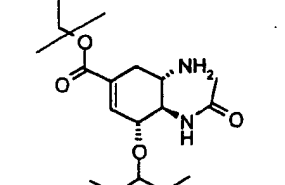
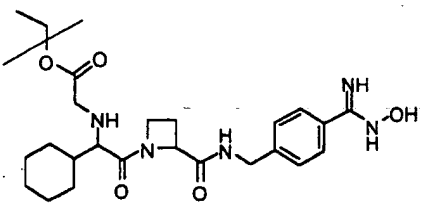
The ACE inhibitor ethyl esters tend to be stable to hydrolysis in human blood and require the action of hepatic (and possibly intestinal and/or kidney) esterases to liberate the active principle [17-20]. Thus, for enalapril the prodrug is 53-74% absorbed following oral dosing in man and the bioavailability of enalaprilat is 36-44%. This is consistent with the release of diacid from ester after intravenous administration, which is calculated to be 60%. The relatively slow hydrolysis of the ethyl ester prodrugs illustrates the potential benefit offered by blood labile prodrugs, which would give even higher concentrations of enalaprilat per mg dose of prodrug. Thus, the oral bioavailability values for individual ACE inhibitors will be dependant on several factors such as intrinsic lipophilicity of the monoester, affinity for the absorptive transporter and the rate of release of the active principles by cellular esterases.

Ximelagatran is a more recent example of an ethyl ester prodrug. It contains two modifications to the direct acting thrombin compound melagatran. In addition to the ethyl ester group, the amidino group of melagatran is hydroxylated in the prodrug. Thus, two metabolic reactions are needed to liberate the active principle [21]. The modifications incorporated into ximelagatran increase the octanol: water partition by 170-fold compared to melagatran. This increased lipophilicity is reflected by an 80-fold greater flux through a

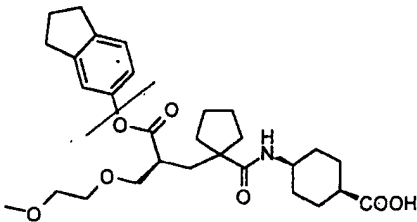
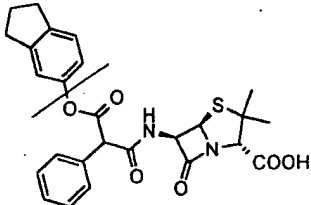
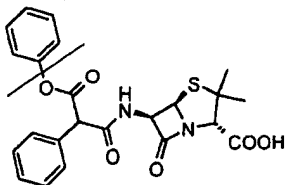
Table 1. Human Absorption and Bioavailability Data for Selected Alkyl and Aryl Ester Prodrugs

Prodrug [references]	Structure	Absorption of prodrug (%)	Oral bioavailability of active principle when given as prodrug (%)
Enalapril [7,19]		53-74	36-44
Benazepril [81]		≥ 37	≥ 17
Quinapril [104]		60	46-52
Cilazapril [8]		≥ 57	57
Moexipril [137, 22]		23	≈ 17.5 estimate from urine moexiprilat excretion after iv and oral moexipril doses
Perindopril [92,105]		ND	19-35 higher values seen in elderly patients
Ramipril [82]		48-56	28-34

(Table 1) contd....

Prodrug [references]	Structure	Absorption of prodrug (%)	Oral bioavailability of active principle when given as prodrug (%)
Spirapril [106]		50-70	42
Trandolapril [93]		≥ 40-60	40-60
Delapril [91,107,108]		35-55	ND
Temocapril [109]		>40	ND
Famciclovir [110]		≥77	54-77
Oseltamivir [111]		>80	79
Ximelagatran [21,22]		ND	18-24

(Table 1) contd....

Prodrug [references]	Structure	Absorption of prodrug (%)	Oral bioavailability of active principle when given as prodrug (%)
Candoxatril [23]		38	32
Carbenicillin indanyl ester [24]		≥ 48-69	48-69 Dose-dependent
Carbenicillin Phenyl ester [24]		>45	41-45

ND = no data

Caco-2 cell monolayer for ximelagatran compared with melagatran [22]. Furthermore, the oral bioavailability of melagatran is 18-24% when dosed as ximelagatran compared to 3-7% when dosed as melagatran [22]. In addition, ximelagatran is unaffected by food whilst the oral bioavailability of melagatran was decreased to 0.9% after food.

ARYL ESTERS

In some cases aryl esters have been preferred to simple alkyl esters (Table 1). Successful prodrugs of this type include candoxatril, and the indanyl and phenyl esters of carbenicillin. The oral bioavailability of candoxatril following oral administration of candoxatril is 32% [23]. Similarly, two aryl esters of carbenicillin have been described. The bioavailability of carbenicillin indanyl ester and carbenicillin phenyl sodium in man is appreciable (> 40%) [24]. Whilst the bioavailability of carbenicillin phenyl sodium is fairly linear with dose the bioavailability of carbenicillin indanyl ester shows clear dose-dependence that is probably due to saturation of an active absorption mechanism. Indeed studies in the rat have shown that carbenicillin indanyl ester is a substrate for an intestinal monocarboxylic acid transport system [25].

DOUBLE ESTERS

Double esters (Table 2) have been the preferred choice for producing oral prodrugs of the β -lactam antibiotics, nucleoside monophosphate anti-viral drugs, for the ACE

inhibitor fosinopril, and for a number of angiotensin II receptor antagonists such as candesartan cilexetil. In most cases where double ester prodrugs have been used clinically, the esters are readily hydrolysed *in vivo* and circulating levels of the intact prodrug are usually not observed.

Morrison *et al.* [26] have conducted a detailed study of the contribution of various organs to the hydrolysis of fosinopril in the dog. In tissue homogenate, the rank order of hydrolysis activity was liver = kidney > small intestine > lung. In blood, the hydrolysis of fosinopril was slow. *In vivo* both the small intestine and liver were shown to have high extraction ratios for fosinopril but due to the intestine having first exposure to the drug it was calculated that 75% of absorbed fosinopril was hydrolysed by the intestine and 25% by the liver.

Sultamicillin is an interesting example of a double ester prodrug where each molecule of pro-drug contains one molecule of ampicillin (an antibiotic) and one molecule of sulbactam (a β -lactamase inhibitor). During the absorption of the prodrug the double ester is cleaved and ampicillin and sulbactam are efficiently delivered to the systemic circulation [27].

CYCLIC CARBONATE PRODRUGS

A further class of clinically precedented prodrugs are the cyclic esters, exemplified by olmesartan and lenampicillin (Table 3). These prodrugs can be considered a subset of the double ester prodrugs. They are designed to be labile in

Table 2. Human Oral Bioavailability Data for a Number of Double Ester Prodrugs. Bioavailability Data of the Active Principle, when Administered as Such, is Given for Comparison where Available

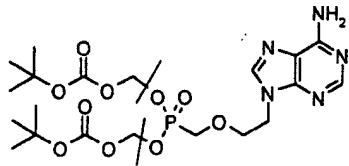
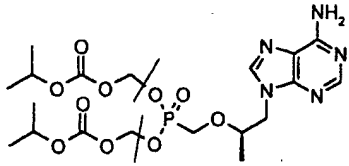
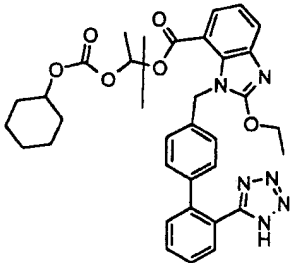
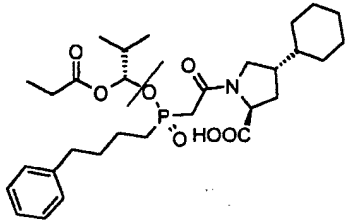
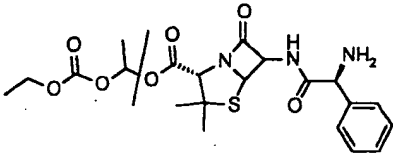
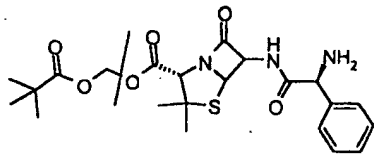
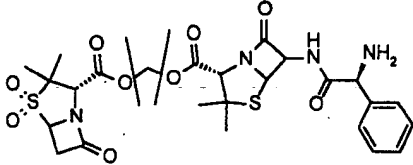
Prodrug	Structure	Oral bioavailability of active principle when given as prodrug (%)	Oral bioavailability of active principle (%)
Adefovir dipivoxil [60,112]		30-45	< 12
Tenofovir disoproxil [136]		25-39 the higher values are in fed subjects	ND
Candesartan Cilexetil [113,114]		42	ND
Fosinopril [88,89,115,116]		23-29	ND
Bacampicillin [43,117,118]		83-89	32-55
Pivampicillin [43,117]		87-94	32-55
Sultamicillin [27,119,120]		> 80 ampicillin > 63 sulbactam	32-55 ampicillin sulbactam has low oral F%

Table 3. Human Oral Bioavailability Data for Examples of Marketed Cyclic Ester Prodrugs

Prodrug	Structure	Oral bioavailability of active principle when given as prodrug (%)
Olmesartan Medoximil [128,129]		26
Lenampicillin [44,130]		= 60

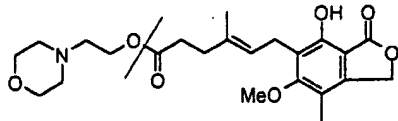
Table 4. Human Oral Absorption Data for Lactone Prodrugs

Prodrug	Structure	Oral absorption of prodrug (%)
Lovastatin [131,132]		30
Simvastatin [131,132]		60-80

Table 5. Human Oral Bioavailability of Amino Acid Prodrugs. Bioavailability of the Active Principle when Dosed Orally is Included for Comparison

Prodrug	Structure	Oral bioavailability of active principle when given as prodrug (%)	Oral bioavailability of active principle (%)
Valganciclovir [28,133]		61	6
Valaciclovir [29]		54	12-20

Table 6. Human Oral Bioavailability of other Marketed Prodrugs

Prodrug	Structure	Oral absorption of prodrug (%)	Oral bioavailability of active principle when given as prodrug (%)
Mycophenolate Mofetil [72]		96.3	93.5

compared to 12 – 20% when aciclovir itself is dosed [29]. The increased oral absorption of these compounds is due to transport by the intestinal dipeptide transporter PEPT1 [30,31]. This transport appears to be stereoselective with the l-valine ester of aciclovir being transported while the d-valine ester is not [29]. Valaciclovir is rapidly converted to aciclovir with the Cmax of valaciclovir being about 10% and the AUC less than 1% of that of aciclovir [29]. In addition, valaciclovir is undetectable in the plasma within 3 hours of an oral dose [32]. The enzyme responsible for hydrolysis of valaciclovir in the rat is located in both the intestine and liver [33].

OTHER ESTERS

Other examples of ester prodrugs used clinically include the morpholinoethyl ester of mycophenolic acid. This prodrug is completely de-esterified with no detectable levels of intact prodrug being observed. Data from intravenous infusion studies suggest that the half-life of the pro-drug is short (< 2 min) with no prodrug being detected within 10 minutes of the end of infusion [34].

ORAL ABSORPTION OF ESTER PRODRUGS

From our review of marketed prodrugs, it is clear that one of the most successful prodrug approaches, the addition of simple esters to polar molecules to improve oral bioavailability, is evident in the ACE inhibitor area. The ACE enzyme is partially responsible for the control of blood pressure and ACE inhibitors have been used in the control of hypertension. ACE is a zinc-dependant di-peptidase that metabolises angiotensin I to the potent vasoconstrictor angiotensin II. Inhibition of ACE decreases the concentrations of angiotensin II at the same time as increasing angiotensin I and renin concentrations. This leads to blood pressure reductions. ACE recognises and subsequently hydrolyses the amide to liberate C-terminal dipeptide of angiotensin I, and inhibitors therefore tend to mimic this polar dipeptide fragment and contain a C-terminal carboxylic acid and a zinc binding group which is a 2nd polar fragment, typically a second carboxylic acid (e.g. enalaprilat), a thiol (e.g. captopril) or a phosphonate (e.g. fosinoprilat). Thus, potent ACE inhibition requires a compound that exhibits high polarity and hydrogen bonding capacity. This is incompatible with extensive transcellular absorption and consequently, the prodrug approach has been employed in order to deliver several ACE inhibitors by the oral route. These include enalaprilat and ramiprilat (Table 1), which are given as the ester prodrugs enalapril and ramipril. Ranadive *et al.* [35] have studied the physicochemical properties of these ACE

inhibitors and their prodrugs. The dicarboxylic acid ACE inhibitors (enalaprilat and ramiprilat) are highly polar molecules (log $D_{(7.0)}$ values of <-3 and -1.96, respectively) and as such are unlikely to be well absorbed by the trans-cellular route. The ethyl esters, enalapril and ramipril, exhibit significant increases in lipophilicity (log $D_{(7.0)}$ values of -1.15 and +0.14, respectively) due to the masking of one of the ionisable carboxylic acid functions. For enalapril, this more than 100-fold increase in lipophilicity leads to a human oral absorption value of 53 to 74% (Table 1). This oral absorption value is unexpected since enalapril is still a polar molecule. However, there is evidence that this ethyl ester is actively absorbed by a carrier mechanism [12,16]. Ramiprilat exhibits higher intrinsic lipophilicity than enalaprilat due to the addition of an extra 3 methylene units. Thus, the oral absorption value for ramipril (54 to 65%) may be driven by increased passive transcellular absorption relative to enalapril.

The phosphorous containing ACE inhibitor, fosinoprilat, is administered as a double ester prodrug (fosinopril), which is attached via the phosphate functionality (Table 2). The log $D_{(7.0)}$ values for fosinoprilat and fosinopril are -0.48 and +2.7 respectively, driven by the masking of the hydroxyl function and the significant addition of the lipophilicity associated with the ester functionality. This approximate 1000-fold increase in overall lipophilicity leads to a human oral absorption value of 32 to 36% for fosinopril and oral bioavailability values of 25 to 29%.

Overall, the ACE inhibitor story suggests that it is possible to improve the oral absorption potential of polar compounds by the prodrug approach. In addition, it provides an insight into the potential problems that may be encountered by the prodrug approach since oral absorption of enalapril, ramipril and fosinopril is not complete. The log $D_{(7.0)}$ values of the ethyl ester derivatives are approximately 0 which suggests equal partition between lipid and aqueous. This leads to significant oral absorption values with the more lipophilic esters exhibiting greater absorption. One could speculate that esters with greater lipophilicity would be likely to exhibit higher oral absorption values in humans. However, simply increasing lipophilicity at the expense of other favourable physicochemical properties is not an attractive option. This is illustrated by fosinopril, which exhibits greater lipophilicity than either of the ethyl esters, but a lower oral absorption value than ramipril. This may be due to the nature of the ester functionality increasing the molecular weight of fosinoprilat towards 500 or the lipophilic nature of fosinopril may lead to a reduction in aqueous solubility and thus potential for solubility limited oral

absorption. Alternatively, the lower oral bioavailability exhibited by fosinopril may be due to non-productive clearance or to a lower contribution of active uptake in the absorption of fosinopril (see later section). Overall however, despite the incomplete oral absorption of these ACE inhibitor prodrugs, they are successful oral antihypertensive agents.

A further example of the prodrug approach to increasing lipophilicity and hence transcellular absorption potential has been provided by Hovgaard *et al.* [36]. These authors studied the effect of the addition of a cyclopropane ester on the lipophilicity and Caco-2 cell flux of a series of β -blocker agents. The results are summarised in Table 7.

Overall, esterification of these β -blocking agents with cyclopropane carboxylic acid increased the $\log D_{(7.4)}$ by over 1 log unit. This led to increases in Caco-2 cell permeabilities. Since Caco-2 cell flux is a good indicator of transcellular absorption potential [37], oral absorption of the esters is likely to be improved over the β -blocking agents, especially for the most polar examples.

The GPIIb/IIIa fibrinogen receptor antagonists provide further good examples of the use of the prodrug strategy. The fibrinogen-platelet interaction is modulated by a 3 amino acid sequence of arginine-glycine-aspartic acid. Antagonists of this receptor need to mimic this amino acid sequence and as such require both acidic and basic centres. Thus, GPIIb/IIIa antagonists are zwitterionic, highly polar and tend to exhibit poor oral absorption.

A prodrug strategy with the GPIIb/IIIa antagonist EMD 80200 Fig. (2) has been reported [38]. The calculated $\log D_{(7.4)}$ of EMD 80200 is -3.2 , due to its basic guanidine and carboxylic acid moieties. This leads to a Caco-2 cell absorptive flux of 0.275×10^{-6} cm/sec, which is incompatible with significant transcellular absorption. In this case, esterification of the carboxylic acid did not lead to an increase in lipophilicity (calculated $\log D_{(7.4)}$ -3.55) or transcellular flux (Papp 0.277×10^{-6} cm/sec) due to the unmasked ionisation potential of the guanidine function. In EMD 122347, this guanidine function was masked with a methyl carbamate moiety, leading to an increase in calculated $\log D_{(7.4)}$ (-1.88 vs -3.2 for EMD 80200). At a concentration of $300 \mu\text{M}$, the Caco-2 cell absorptive flux of EMD 122347 was 7.3×10^{-6} cm/sec, some 25-fold greater than EMD 80200. However, flux in the non-absorptive (secretory) direction was 13.4×10^{-6} cm/sec. In addition, absorptive flux was shown to be saturable with increasing

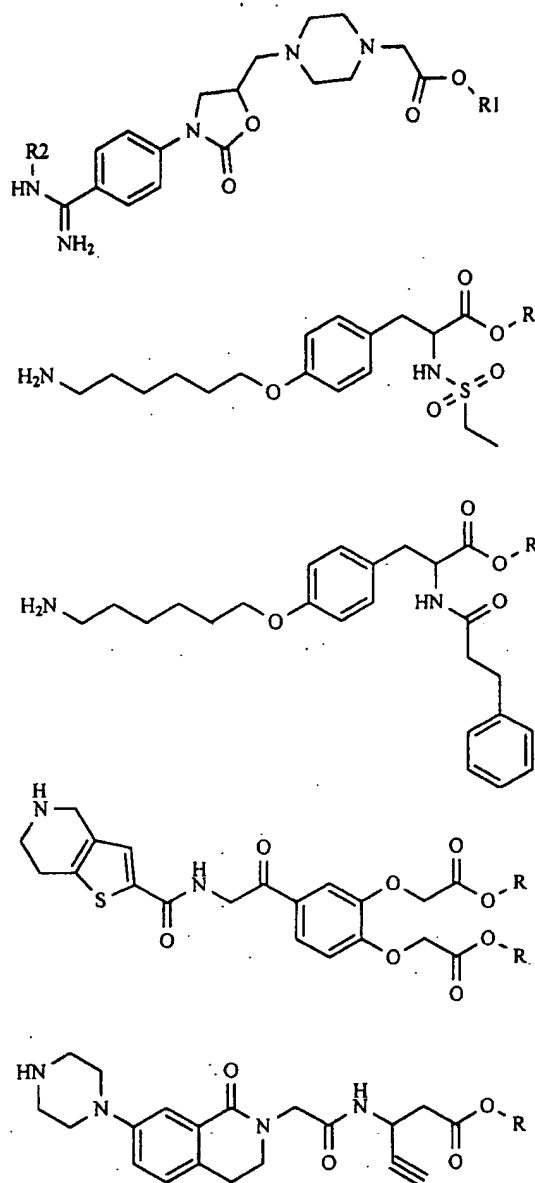
concentration of EMD 122347 and the efflux ratio could be abolished with verapamil. This profile of Caco-2 cell monolayer flux has been described previously [39, 40] and is ascribed to saturable efflux by drug transporters (such as P-glycoprotein). Interestingly, the asymmetry of flux was not observed with more lipophilic prodrugs of EMD 80200, where the ethyl ester was retained and greater lipophilicity was added at the guanidine end of the molecule. Overall, the increased Caco-2 cell flux of EMD 122347 over EMD 80200 was shown to translate to increased oral absorption as judged by oral bioavailability determination in cynomolgous monkeys (EMD 80200 3 to 10% and EMD 122347 40%). However, the more lipophilic esters, although showing greater Caco-2 cell flux, did not exhibit greater oral bioavailability values in the monkey. This may have been due to non-productive clearance of the prodrug.

A further example of efflux transporters limiting the absorptive flux of prodrugs in the Caco-2 cell flux model of oral absorption has been described [41]. The fibrinogen receptor antagonist, LY-767,679, is a polar ($\log D_{(7.4)} < -3$) and zwitterionic compound which exhibits low oral bioavailability in animals. Caco-2 cell flux of LY-767,679 was low (0.25 to 0.5%/h in both directions) indicating that this low oral bioavailability was due to poor absorption. The benzylic prodrug (LY-775,318, Fig. (2)) was significantly more lipophilic ($\log D_{(7.4)}$ 0.7) than LY-767,679. However, this did not lead to a large increase in absorptive Caco-2 flux (0.75%/h). In addition, flux in the secretory direction was 3%/h and this asymmetry could be abolished with verapamil, suggesting that P-glycoprotein was limiting the flux of this prodrug. Consequently, P-glycoprotein would be likely to limit the *in vivo* oral absorption of LY-775,318 and is a potential barrier to any prodrug approach.

One important area that has benefited from prodrug oral delivery is the antibiotics. Ampicillin is a polar antibiotic, which exhibits incomplete oral absorption [42]. This was improved by the synthesis of a pivaloyloxymethyl ester (pivampicillin, Table 2). In rats, at 2 hours following a 100mg/kg oral dose of ampicillin, the plasma concentration of ampicillin was $0.84 \mu\text{g/ml}$. An equivalent dose of pivampicillin produced a 2h ampicillin plasma concentration of $4.9 \mu\text{g/ml}$. In dogs, a similar observation was made following 30mg/kg equivalent doses producing 2 and $5.7 \mu\text{g/ml}$ concentrations of ampicillin, respectively. In humans, urinary excretion of ampicillin was improved from 49% to 70% [43] when pivampicillin was given orally,

Table 7. $\log D_{(7.4)}$ Values and Caco-2 Cell Permeability Coefficients for a Series of β -Blocker Agents

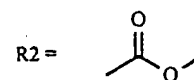
Compound	$\log D_{(7.4)}$	$\log D_{(7.4)}$ of ester	Caco-2 cell Papp ($\times 10^{-6}$ cm/sec)	Caco-2 cell Papp ($\times 10^{-6}$ cm/sec) of ester
Acebutolol	-0.04	1.59	4.5	78
Alprenolol	0.31	2.78	75	108
Betaxolol	0.28	0.63	49	97
Oxprenolol	0.45	1.98	66	97
Propanolol	1.38	3.0	82	104
Timolol	-0.04	1.74	44	79



EMD80200 R1 = H R2 = H

EMD85900 R1 = Ethyl R2 = H

EMD122347 R1 = Ethyl



RGD Peptidomimetic 1a R = H

RGD Peptidomimetic 1b R = Methyl

RGD Peptidomimetic 2a R = H

RGD Peptidomimetic 2b R = Methyl

ME3277 R = H

ME3229 R = n-Butyl

LY-767,679 R = H

LY-775,318 R = Benzyl

Fig. (2). Structures of GPIIb/IIIa receptor antagonists and prodrugs.

suggesting that substitution of pivampicillin for ampicillin leads to an improvement in oral absorption. A similar observation was made in mice and humans with the 5 methyl-(2-oxo-1,3, dioxolen-4-yl methyl) cyclic ester of ampicillin (KBT-1585, lenampicillin, Table 3, [44,45].

One potential pitfall of the prodrug approach is highlighted by the cephalosporins. Active cephalosporins tend to be polar acids, which suffer from poor oral absorption. Nishimura *et al.* [46] have studied the effect of mono- and bi-functional prodrugs of a polar cephalosporin, KY087 Fig. (3). KY106 is a (5-methyl-2-oxo-1,3-dioxolen-4-yl) methyl ester of KY087 which increases the log $D_{(6.5)}$ from -2.0 to +1.6. Although this lead to an increase in oral absorption (C_{max} values at equivalent doses in rats were 2.5 fold higher than for KY087) the prodrug was more slowly absorbed. This was ascribed to solubility-limited oral absorption. Indeed, when KY106 was further functionalised with the addition of alanine to produce the more soluble bi-

functional prodrug, KY109, the oral absorption in rats was more rapid and extensive when compared with KY106 (T_{max} 30 minutes and C_{max} 4-fold higher). Thus, the balance of lipophilicity and aqueous solubility is an important consideration in the design of an oral prodrug. A similar approach to KY109 was employed for ceftizoxime [47]. Following oral administration to rabbits, the mono-functional pivaloyloxymethyl ester of ceftizoxime Fig. (3) improved the urinary recovery of ceftizoxime from 5.4 to 32%. However, at pH 6.0, the aqueous solubility was 0.38mg/ml. Derivatisation with alanine at the aminothiazole moiety produced AS-924 with a log $D_{(6.5)}$ of 1.15 and an aqueous solubility of 4.3mg/ml at pH 6.0. This led to a further increase in urinary recovery of ceftizoxime to 43%.

With other cephalosporins different approaches have been taken to address the balance of lipophilicity and aqueous solubility. Yoshimura *et al.* [48] have synthesised ester prodrugs of the zwitterionic cephalosporin, cefotiam

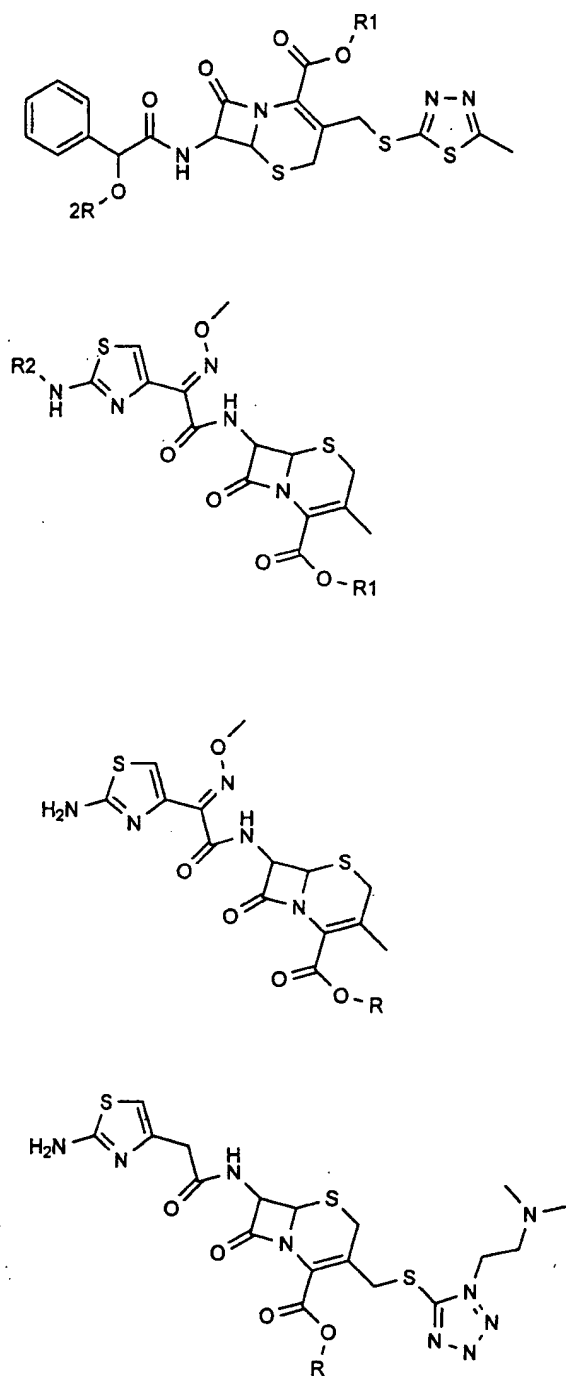


Fig. (3). Structures of cephalosporins.

Fig. (3). The propylvaleryloxymethyl and ethylvaleryloxymethyl esters improved the oral bioavailability of cefotiam in mice from 6% to 54 and 46%, respectively. The aqueous solubility of these esters is unlikely to be limiting oral absorption due to the presence of the basic centre. A further method to maintain a basic centre in a prodrug cephalosporin was outlined by Kakeya *et al.* [49]. In this instance, the ester functionalities used contained basic centres. These prodrugs improved the oral bioavailability of the cephalosporin Fig. (3; ester 2a-c) from approximately 3% to 15 to 41%.

KY087 R1 = H R2 = H

KY106 R1 = R2 = H

KY109 R1 =

KY109 R2 =

Ceftizoxime R1 = H R2 = H

AS-924 R1

AS-924 R2

Parent Cefalosporin R = H

Ester 2a R =

Ester 2b R =

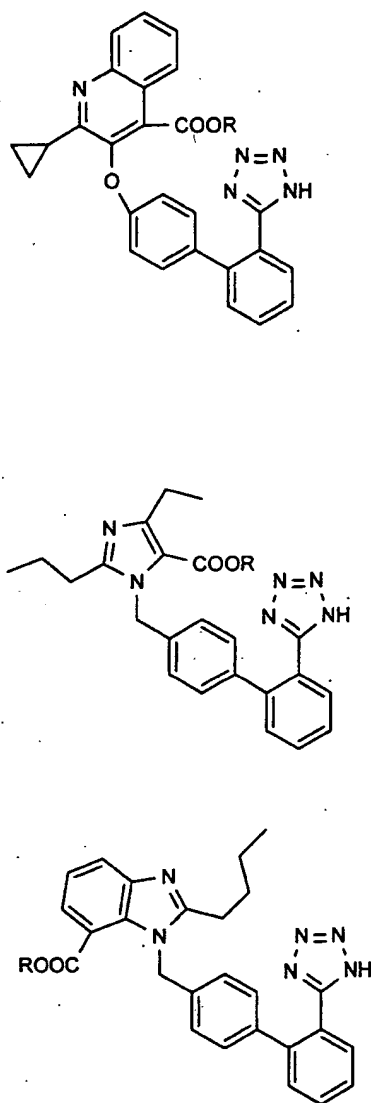
Ester 2c R =

Cefotiam R = H

Ester 1 R =

Ester 2 R =

The angiotensin II receptor antagonist drugs tend to be diacidic, with the result that the polarity precludes good membrane permeation and hence oral absorption. Thus, most of this class of drugs are administered as prodrugs. The oral bioavailability of BMS-183920 Fig. (4) in rats is 11%. Obermeier *et al.* [50] have shown that this compound exhibits low membrane permeability in Caco-2 cells (Papp 0.9×10^{-6} cm/sec) consistent with poor oral absorption. A series of carboxylic ester prodrugs showed that improved Caco-2 cell flux could be obtained (Papp values ranging



BMS183920 R = H

Prodrug 2 R =

Prodrug 3 R =

Prodrug 4 R =

Prodrug 5 R =

Prodrug 6 R =

DMP811 R = H

Prodrug R =

CV-11194 R = H

Prodrug 1 R =

Prodrug 2 R =

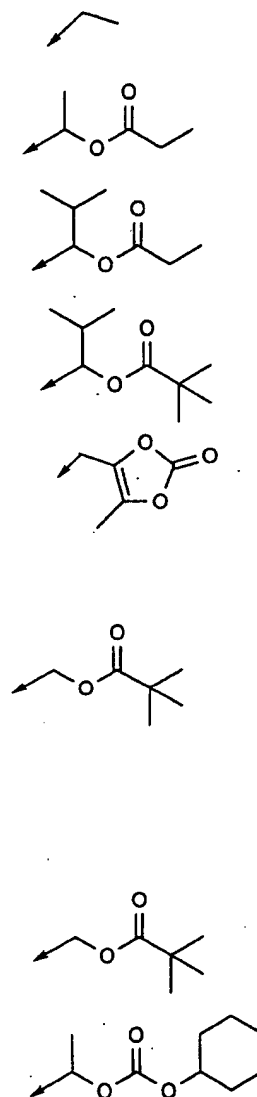


Fig. (4). Structures of Angiotensin II receptor antagonists.

from 4.4 to 13.5×10^{-6} cm/sec) and this lead to improvements in oral bioavailability of up to 2-fold. Similar observations were made with the pivaloyloxymethyl esters of DMP811 (4-fold increase in oral bioavailability in rats over DMP811, [51]) and CV11194 (10-fold increase in oral bioavailability in rats over CV11194) [52,53].

Another successful prodrug approach is that of adefovir dipivoxil (Table 2). Adefovir is an important phosphorus-containing nucleotide analogue antiviral agent. Due to its polar nature, adefovir exhibits 8% oral bioavailability in rats [54], 4% in cynomolgous monkeys [55] and less than 12% in humans [56], limited by poor oral absorption.

Adefovir exhibits very low flux (<0.1%/h) in the Caco-2 cell monolayer model [57,58], due to its high polarity (log P -4.11). The mono-(pivaloyloxymethyl)-ester exhibits a log P of -2.95 whereas the bis-ester (both phosphonate hydroxyl groups masked) exhibits a log P of +2.48. In common with adefovir, the mono-ester is poorly fluxed in Caco-2 cells and

would not be considered a viable prodrug. When the bis-ester was applied to Caco-2 cells, there was a high rate of hydrolysis to both the monoester and adefovir. Analysis of the basolateral compartment suggested that the transport of 'total' adefovir was approximately 9%/h made up of 2, 4 and 3%/h transport of the bis-ester, the monoester and adefovir, respectively. This flux was also concentration dependent since when the apical concentration of the bis-ester was lowered to 25 μ M from 100 μ M, the flux of 'total' adefovir was reduced to approximately 1%/h, made up of approximately 0.8%/h adefovir and approximately 0.2%/h mono-ester. In addition, following loading of the Caco-2 cells with the bis-ester, a significant proportion of the compound was effluxed back into the apical compartment as both the mono-ester and adefovir, have a 6-fold asymmetry over the flux in the basolateral direction.

Overall, these experiments suggest that the bis-(pivaloyloxymethyl)-ester of adefovir (now marketed as adefovir

dipivoxil) exhibits greater oral absorption potential than both adefovir and the monoester due to increased lipophilicity. However, absorption of adefovir dipivoxil is unlikely to be complete due to hydrolysis of the ester functionalities during absorption combined with efflux of the products back into the gut lumen.

Indeed, adefovir dipivoxil does show improved oral bioavailability relative to adefovir in rats (38% versus 8%, [59]), cynomolgous monkeys (25% versus 4%, [55]) and humans (30 to 40% versus <12%, [60]). However, since adefovir is extensively excreted in the urine (ie metabolism is minimal), these values suggest that oral absorption is not complete, probably limited by intestinal hydrolysis and transporter-mediated efflux.

Tenofovir is a further example of a nucleoside phosphonate compound that is administered orally as a prodrug containing two double ester prodrug moieties (Table 2). This prodrug dramatically increases the oral bioavailability of the monophosphonate in mice when compared to oral dosing of the monophosphonate directly (20 vs 2%) [61]. Moderate oral bioavailability has also been achieved in the dog (30%) and in human (27-41%) using this prodrug. Experiments *in vitro* showed that the prodrug was metabolised by a number of canine tissues with the following rank order of metabolism liver > plasma > intestine [62]. Minimal intact prodrug was observed in the plasma of pre-clinical species after oral dosing [136].

Clearly, the prodrug approach is a viable strategy for increasing the oral absorption of polar molecules, which has been successful on many occasions. However, this route is not an easy option versus obtaining optimal absorption of the active principle. Several factors need to be taken into consideration. The rationale for the prodrug approach is to increase lipophilicity to improve membrane permeation. However, this will also lead to reduction in aqueous solubility and so a balance needs to be struck. On several occasions, masking polar functions with ester functions has lead to affinity for various efflux transporters that can reduce the absorption potential of a molecule. In addition, ester hydrolysis can occur during the absorption process, which can also reduce the overall absorption of a molecule, especially if the hydrolysis products are substrates for efflux transporters. Consequently, the prodrug strategy almost invariably does not lead to complete oral bioavailability of the active principle and many factors need to be taken into consideration at the outset. For this reason, we propose that such a prodrug strategy is only considered when all other attempts to modify the physicochemistry of the active principle to increase the oral absorption have failed. For those approaches with an absolute requirement to mount a prodrug strategy, we can now outline the problems likely to be encountered by the Discovery scientist.

CHEMICAL CONSIDERATIONS FOR ESTER PRODRUGS

There are four main chemical factors to be taken into account when designing an ester prodrug:

1. Sufficient stability to enable synthesis, purification and formulation of drug substance.

2. Sufficient aqueous solubility to allow complete oral absorption.
3. Greater bioconversion rate in tissue or blood compared to hydrolysis in aqueous solution at physiologically relevant pH.
4. The liberated fragments of the hydrolysis must have low toxicity and no unwanted pharmacology.

When present in a commercially viable pharmaceutical product, an ester needs to be sufficiently stable to enable the chemical synthesis, isolation and purification of the drug substance. This material also needs to be stable to formulation into the final drug forms, such as tablets, capsules, syrups, solutions (aqueous and organic) and ointments, and with sufficient stability to have an appropriate 'shelf-life'.

Simple alkyl esters, particularly ethyl esters, are commonly used as prodrugs. The stability profile of these esters is generally good over the pH range of approximately pH4 to pH9, with hydrolysis occurring rapidly outside of this range. There are 8 possible mechanisms for acid- and base-catalysed ester hydrolysis [63], however in practice only 2 are commonly found. These are AAC2 for most acid-catalysis and BAC2 for almost all base-catalysed hydrolysis. The main difference between hydrolysis in alkali or acid is in base hydrolysis the powerful nucleophile OH⁻ attacks the carbonyl carbon, whilst in acid-hydrolysis a proton (H⁺) makes the ester more positive, and therefore susceptible to addition of water to make a tetrahedral transition state Fig. (5). It is possible to increase or decrease stability by steric or electronic influences. For example, the rate-limiting step in BAC2 alkali hydrolysis is nucleophilic attack of hydroxide anion at the ester carbonyl. Sterically hindering this attack, by introducing branching, increases alkaline stability. Thus, *t*-butyl is more stable than *i*-butyl, which in turn is more stable than *n*-butyl.

In parallel with significant chemical stability, the simple alkyl esters of some parent acid drugs have such low bioconversion rates *in vivo* that the primary clearance mechanism for the prodrug is not esterase mediated. This is not ideal for the prodrug approach (see Non-productive metabolism section). The penicillin class of antibiotics are good examples, as their simple alkyl and aryl esters are not hydrolysed *in vivo* and so the introduction of double esters was used to increase the conversion rate [64]. These double esters shift the recognition and centre for hydrolysis to the second ester. Thus, esterases can recognise and hydrolyse this additional ester and liberate parent acid together with the linking aldehyde fragment Fig. (6). However, the intrinsic chemical stability of these double esters is low and this approach is generally reserved for when the simple esters are unacceptably resistant to bioconversion. It is possible to increase the chemical stability of these double esters with alkyl groups on the linking methylene. In addition, the increased lipophilicity, which this affords, helps increase absorption potential. The other benefit of this substitution is that it avoids liberating toxic formaldehyde, with alternatives such as acetaldehyde being more toxicologically acceptable. However, a chiral centre is introduced which increases complexity as 2 distinct pharmacokinetic profiles of the enantiomers may be observed. The toxicity of the liberated

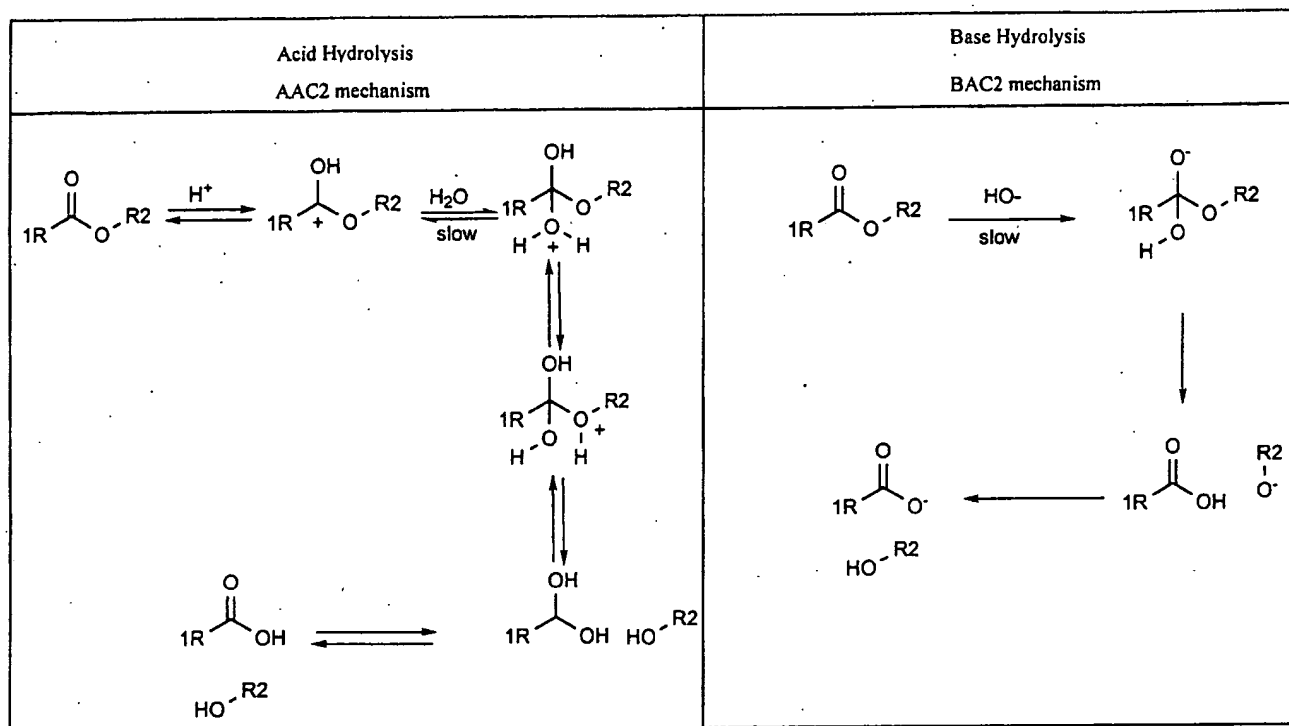


Fig. (5). Mechanisms of alkyl ester hydrolysis.

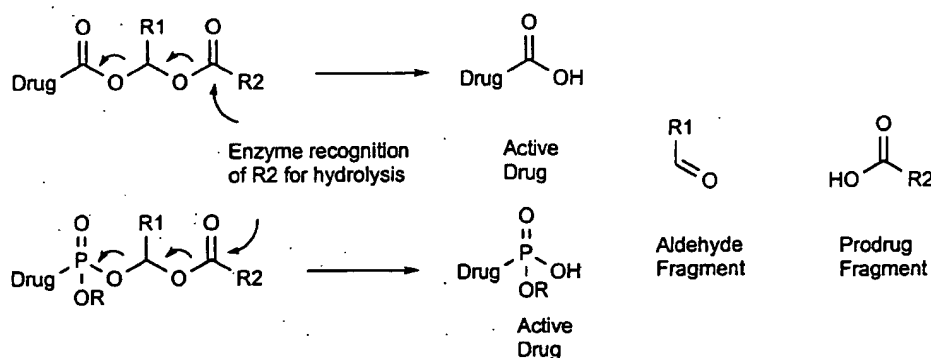


Fig. (6). Mechanism of double ester hydrolysis.

prodrug acid fragment also needs to be taken into consideration, and as an example pivalic acid (trimethylacetic acid) was once commonly used but is now associated with some degree of toxicity [65]. More recent double ester prodrugs avoid pivalic acid. In the case of the angiotensin receptor antagonist candesartan cilexetil (Table 2) a ($\pm$)-1-[[[(cyclohexyloxy)carbonyl]oxy]ethyl ester was selected [52], as the single alkyl esters of candesartan were stable to bioconversion. As the recognition site is remote to the drug carboxylic acid this approach can also be used to prodrug other polar functional groups, such as the phosphonic acid (e.g. adefovir dipivoxil).

As discussed in other sections, there is a great benefit to be found if an ester is hydrolysed in the blood or plasma. Several attempts have been made to identify prodrug fragments that are substrates for blood borne enzymes. One very successful approach is the use of (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl cyclic esters, which avoids

introducing a chiral centre Fig. (7). These were shown to be plasma labile, and found utility as antibiotic prodrugs, for example the ampicillin prodrug KBT-1585 (lenampicillin, Table 3) [45]. Recently, it has been shown that this group is a substrate for the plasma enzyme, paraoxonase [66, 67]. The mild alkaline hydrolysis liberates a diketone, although this has not been substantiated as a product in the paraoxonase mediated hydrolysis. A further example using this ester is olmesartan medoximil [68].

A simpler approach to designing blood labile esters involves substitution of simple alkyl ester with amides or amines. Using benzoic acid as a model 'drug', Nielsen and Bundgaard [69] showed glycolamide esters to have remarkably high hydrolysis rates in plasma compared to alkyl esters, with only a modest increase in intrinsic hydrolysis rates in aqueous buffers. The enzymes responsible for the hydrolysis *in vivo* are believed to be butyrylcholinesterases. Additionally, these enzymes also recognise

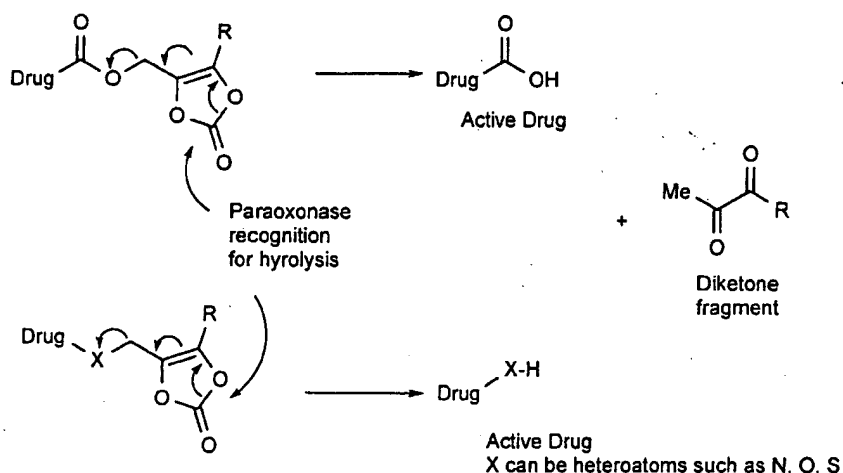


Fig. (7). Mechanism of cyclic carbonate ester hydrolysis.

aminoethyl esters. Mørk and Bundgaard [70] subsequently prepared prodrugs of ibuprofen and showed a large increase in plasma hydrolysis rates of both aminoethyl and glycolamide esters compared to the ethyl ester (Table 8).

An example of a marketed glycolamide ester is camostat mesylate Fig. (8), which has a half-life of less than 1 minute in human plasma [71]. The aminoethyl prodrugs are also exemplified by mycophenolate mofetil (Table 6 and Fig. (8)) where a morpholinoethyl ester is used to give a plasma hydrolysis half-life of a few minutes [72]. An additional advantage of aminoethyl esters is the increased solubility gained by the presence of a basic amine, whilst the relatively low pKa of the aminoethoxy moiety means that a proportion of unionised prodrug is present for transcellular flux.

As previously stated, lactones are used as a method for increasing the oral absorption of HMG-CoA reductase inhibitors. Lactones are unique in that the hydrolysis is reversible and it is possible to establish equilibrium in plasma [73] between the 2 forms Fig. (9). The free hydroxy

acids have a tendency to spontaneously lactonise and are kept as salts, such as sodium or calcium salts, to prevent this cyclisation. It is more convenient to administer the more stable and permeable lactones as prodrugs of the active drug.

NON-PRODUCTIVE CLEARANCE OF ESTER PRODRUGS

The major aim of the prodrug approach is to produce high concentrations of the active principle in the systemic circulation. In the case of ester prodrugs, this means that quantitative ester hydrolysis to the active principle is the ideal. However, the ester prodrug is exposed to a wide variety of metabolic and transporter-mediated processes on passage from the g.i. tract to the systemic circulation. The extent to which the ester avoids these alternative processes and is metabolised by esterases, will determine the potential success of a prodrug approach. As has been stated previously, the majority of ester prodrugs do not express complete oral bioavailability. In some cases, this may be due

Table 8. Hydrolysis Rates of a Series of Ibuprofen Esters in Human Plasma

R = H (R-Ibuprofen)	Half-Life values in 80% human plasma at pH 7.4 and 37°C (mins)
	5580
Glycolamide	1.5
:HCl Aminoethyl	1.7

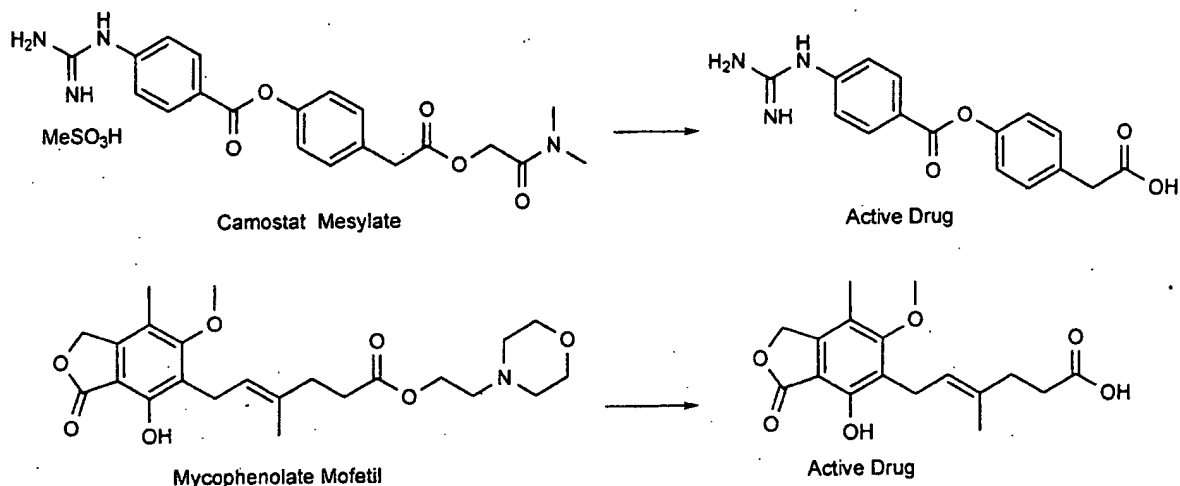


Fig. (8). Aminoalkyl- and amidoalkyl-esters.

to incomplete oral absorption, but for a significant number, the loss of active principle may well be due to clearance by non-esterase mechanisms.

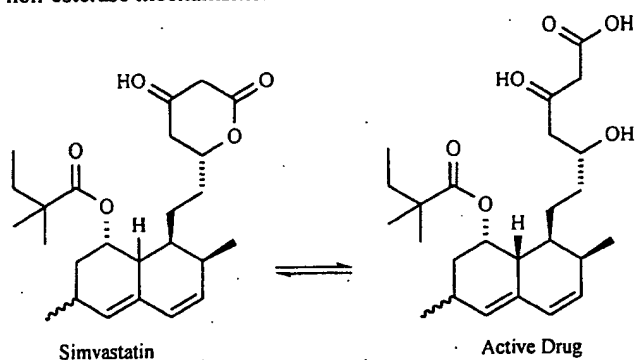


Fig. (9). Inter-conversion of lactone and active drug forms of simvastatin.

These mechanisms fall into two broad categories:

- 1) Non-productive ester hydrolysis where the prodrug is metabolised by an esterase but this does not lead to liberation of active principle into the systemic circulation. This includes:
 - (a) Hydrolysis of the prodrug in the gut lumen.
 - (b) Hydrolysis of the prodrug in the intestinal cell during absorption combined with return to the gut lumen.
 - (c) Hydrolysis of the prodrug in the liver combined with excretion into the bile.
 - (d) Further metabolism of the released active principle post ester hydrolysis and prior to release into the systemic circulation.

- 2) Metabolism of the prodrug by a non-esterase enzyme.

Each of these will be discussed in turn.

NON-PRODUCTIVE HYDROLYSIS IN THE INTESTINES (A AND B)

Non-productive hydrolysis can occur prior to absorption due to instability of the ester in the intestinal contents. If

instability in the intestine is high, only the active principle will be available for absorption and the prodrug will provide no benefit. This emphasises the importance of chemical stability in the design of ester prodrugs (See chemical considerations section). Such intestinal instability has been observed for desmopressin prodrugs, which are hydrolysed in rat jejunal fluid [74].

The intestinal wall cells contain a significant amount of esterase activity [3]. Thus, a prodrug can be subject to hydrolysis during the absorptive process. Clearly, if the active principle liberated within the cell passes through the basolateral membrane into the blood, then the prodrug will have delivered active principle. Since, the aim of the prodrug approach is to improve passive membrane permeability, it is likely that passage of the active principle through the basolateral membrane will be transporter mediated. As transporter proteins are expressed at both the basolateral and apical membranes of the gut wall cell, the active principle is as likely to be effluxed back into the gut lumen as it is to proceed into the blood. Consequently, intestinal ester hydrolysis of a prodrug is likely to lead to a reduction in oral bioavailability. Indeed, the literature suggests that apical efflux of released hydrolysis products is greater than passage across the basolateral membrane.

Camenisch *et al.* [75] have examined the esterase liability of a series of RDG peptidomimetics Fig. (2). The active principle peptidomimetics were poorly absorbed following oral administration due to a high degree of polarity. In Caco-2 cell flux experiments peptidomimetics 1a and 2a exhibited poor flux. The lipophilicity of the respective methyl esters (1b and 2b as determined by Log Kw) was approximately 100-fold greater than their respective active principles. In the absorptive direction there was extremely limited appearance of the intact prodrug in the basolateral compartment, suggesting poor absorptive flux. However, there were high concentrations of the active principles in the basolateral compartment. Thus, the prodrugs improve entry into the Caco-2 cells where ester hydrolysis occurs. The active principles can progress into the basolateral compartment. Overall absorptive flux of 'total' peptidomimetics (active principle and prodrug) was significantly higher than that of the active principle only. In addition, when experiments were

repeated in the secretory direction, flux of 'total' peptidomimetic was approximately 4-fold greater than in the absorptive direction. Again this suggests that efflux transporters may have a role to play in modulating the oral absorption potential of these peptidomimetics and their methyl ester prodrugs.

Okudaira *et al.* [76,77] have further elucidated this for another GPIIb/IIIa receptor antagonist. ME3277 Fig. (2) is a very polar compound ($\log D_{(7.4)} -3$) due to two acidic and one basic centres. It exhibits 1% oral bioavailability in rats and the Caco-2 cell absorptive Papp of 0.085×10^{-6} cm/sec suggests that this is due to poor oral absorption. When the carboxylic acid groups of ME3277 were masked with ester functions, the resultant prodrug (ME3229) exhibited a $\log D_{(7.4)}$ value of 1.3. In Caco-2 cells, the absorptive flux of 'total' ME3229 (ME3229 and all de-esterified metabolites) was 7.9×10^{-6} cm/sec, suggesting a significant absorption potential of the ester prodrug. The major drug-related species in the basolateral compartment was the monoester of ME3277, suggesting hydrolysis had occurred during transport.

Despite the improved transcellular flux observed in the Caco-2 cell model of absorption, the oral absorption of ME3229 was only 10%. In single pass perfusion experiments with rat small intestine, the disappearance rate of ME3229 was 76-fold greater than the appearance rate in the mesenteric blood. Further experiments suggested that in part this was due to pre-absorption degradation by esterases present in the gut lumen. When ME3229 was withdrawn from the perfusate, the mono-ester metabolites continued to appear. This suggests that ME3229 passes into the gut wall cell, is metabolised to the monoesters, which are then actively effluxed back into the gut lumen. Thus, although ME3229 represents a significant improvement in cell permeation over ME3277, the improvement in oral bioavailability is marginal due to extensive hydrolysis before and during absorption and active efflux of the monoester metabolites back into the gut lumen.

NON-PRODUCTIVE HYDROLYSIS IN THE LIVER (C AND D)

Prodrugs that rely on hepatic esterases for release of the active principle, face a series of issues in terms of optimising oral bioavailability. The first issue relates to the physicochemistry of the active principle. The aim of the prodrug approach is to improve the membrane permeability of a polar molecule. Hepatic esterases release the active principle inside a membrane-enclosed hepatocyte. Thus, in order to maintain high concentrations in the systemic circulation, the active principle must cross the hepatocyte membrane. This is most likely to be an active process. However, many transport proteins are expressed at the canalicular membrane. Thus, if a released active principle is a preferential substrate for a canalicular transport protein, it will be secreted into the bile and excreted, rather than deposited into the blood. This will manifest as an attenuation of oral bioavailability. This is exemplified by the ACE inhibitor, temocaprilat (the active principle of the prodrug temocapril, Table 1), [78], which is excreted in to bile via the canalicular multispecific organic anion transporter (cMOAT). Also following administration of enalapril,

perindopril and ramipril to the isolated perfused rat liver 46, 27 and 71% of the administered dose was excreted as the diacid into bile, indicating the potential importance of this route of elimination [79].

The second issue facing active principle released inside the hepatocyte is the array of metabolic enzymes present. Any further metabolism of the released active principle will result in lower than expected oral bioavailability. For examples of this, we return to the ACE inhibitors where it has shown been shown that both benazeprilat [80,81] and ramiprilat [82] form glucuronide metabolites as a clearance mechanism. Additionally, ramiprilat is further metabolised to a diketopiperazine, via the acyl glucuronide promoting intramolecular attack and cyclisation Fig. (10).

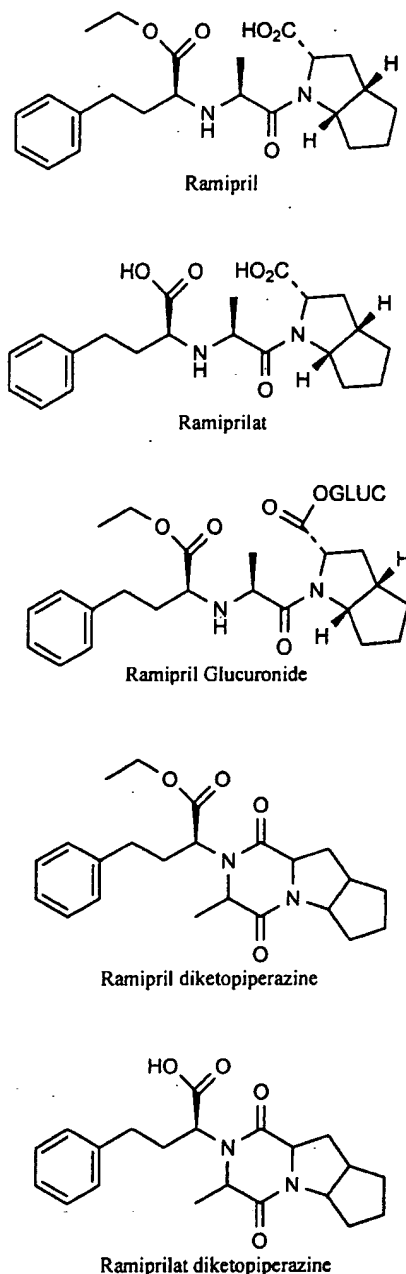


Fig. (10). Non-productive metabolism of ramipril.

Finally, a prodrug strategy must consider the potential for the prodrug itself to undergo non-productive clearance. As well as enhancing the oral absorption of a compound the increased lipophilicity of the prodrug may lead to a greater potential for non-ester metabolism or other clearance pathways. Therefore, the increased lipophilicity to promote absorption must be balanced against the ester hydrolysis rate and non-productive clearance by other mechanisms, which would attenuate oral bioavailability.

There are a number of examples of this, most notably in the ACE inhibitor arena. Enalapril and enalaprilat exhibit differences in their metabolic profile in the rat. *In vitro* experiments with rat hepatocytes have shown that at high concentrations (0.5-4 mM) enalapril is metabolised to a cytotoxic metabolite by rat CYP 3A1/2, whereas enalaprilat even at concentrations of 10mM was not toxic to the rat hepatocytes [83]. It has been proposed that this difference is due to a relative inability of the more polar enalaprilat to enter the hepatocyte. Furthermore in the rat, Drummer and Kourtis [84] have identified 2-N-alanyl-4-phenylbutanoic acid, a hydrolytic metabolite of enalapril, as a significant urinary excreted metabolite (9% of dose following oral administration of enalapril). Additionally, in the once through isolated perfused rat liver, Pang *et al.* showed that there were large differences in the biliary excretion of enalaprilat when the enalaprilat was generated *in situ* in the liver from enalapril (18% of dose) compared to when enalaprilat was itself administered to the IPRL (5% of dose) [85]. There was also a large difference in the steady state hepatic extraction of enalapril (0.86) compared with enalaprilat (0.05) [85]. In further studies by this group [86], it was proposed that there was a diffusional barrier that prevented the more polar enalaprilat from entering the hepatocyte. Furthermore it was proposed that once formed intracellularly the polar nature of enalaprilat prevents its rapid efflux into the perfusate (blood) and the polar metabolite is available for excretion into bile at a higher rate.

Enalapril is 53-74% absorbed in man (Table 1), which leads to an oral bioavailability of enalaprilat of approximately 40%. The conversion occurs primarily in the liver and appears to be approximately 60% efficient. Thus, 40% of the absorbed dose of enalapril does not yield enalaprilat. It is tempting to speculate that this is due to metabolism of enalapril or biliary elimination of enalaprilat. However, no further metabolites of enalapril or enalaprilat have been identified in humans suggesting that the latter is more likely [19,87].

Ramipril and fosinopril are more lipophilic ACE inhibitor prodrugs than enalapril. Hui *et al.* described the most complete study of human metabolism of fosinopril in patients with mild renal impairment [88]. In this study the metabolism and pharmacokinetics of intravenously administered [^{14} C]-fosinoprilat and orally administered [^{14} C]-fosinopril were investigated. Following intravenous dosing of fosinoprilat, 41% of the dose was recovered in the urine and 44% in faeces. Fosinoprilat constituted 90% of the radioactivity in urine, p-hydroxyfosinoprilat 8% and fosinoprilat acyl glucuronide 1%. In faeces, 75% of the radioactivity was due to fosinoprilat and 5% to p-hydroxyfosinoprilat. Following oral dosing, 10% of the radioactivity was recovered in the urine and 76% in faeces. Fosinoprilat constituted 67% of

the radioactivity in the urine, p-hydroxyfosinoprilat 29%, fosinoprilat acyl glucuronide 2% and fosinopril 0.3%. In the faeces, 72% of the radioactivity was due to fosinoprilat and 16% to p-hydroxyfosinoprilat. Similar results were observed in healthy volunteers [89] and in patients undergoing hemodialysis [90]. In the study by Gehr *et al.* [90], the plasma concentrations of fosinoprilat and metabolites were also measured. One hour after an intravenous dose of fosinoprilat 94% of the radioactivity was due to fosinoprilat and 2% was due to the p-hydroxy metabolite. Two hours after an oral dose of fosinopril, fosinoprilat accounted for only 74% of the plasma radioactivity with substantial amounts of acyl glucuronide (13% of radioactivity) and with some p-hydroxyfosinoprilat (2% of radioactivity) being observed. These data suggest that fosinopril is subject to a greater non-productive clearance than fosinoprilat, and that this contributes to the observed incomplete oral bioavailability of 25 to 29% in humans.

A similar observation has been made for ramipril [82]. When ramiprilat was administered intravenously to humans 67% of the dose was excreted in the urine mainly as unchanged ramiprilat. A small amount of ramiprilat glucuronide was also present. When ramipril was administered intravenously 49% of the dose was excreted in urine and again ramiprilat was the major metabolite present. However, following ramipril administration significant amounts of both ramipril and ramiprilat glucuronide and ramipril and ramiprilat diketopiperazine were observed. Suggesting that the lipophilic prodrug ramipril is metabolised to a greater extent than the more hydrophilic diacid. This may reflect a barrier to entry of the more polar diacid into metabolically active organs such as the liver. When ramipril was administered orally to humans only 23% of the dose was excreted into the urine. The major metabolite was the diketopiperazine acid, with ramiprilat accounting for only 4% of the dose. This suggests that ramipril undergoes significant first pass metabolism in the gut and liver.

Other examples of lipophilic ACE inhibitor prodrugs forming metabolites other than the active principle include delapril that is metabolised to a hydroxy metabolite and to a diketopiperazine [91]. In addition, a glucuronide metabolite of perindoprilat has been identified [92]. Moexipril forms two diketopiperazine compounds of the ester and moexiprilat. Trandolapril is metabolised to two glucuronide conjugates (1 of ester; 1 of trandolaprilat) and two diketopiperazine metabolites (1 of ester; 1 of trandolaprilat) [93].

In addition, angiotensin II receptor antagonist BMS-183920 (Fig. (4)) provides another example of the competing roles of ester hydrolysis and metabolism in determining oral bioavailability. Following intravenous administration to rats, BMS-183920 was poorly metabolised with 91% of the dose being recovered unchanged in the bile of bile duct cannulated rats during the first 12 hours post-dose [50]. The oral bioavailability of BMS-183920 in the rat was 11%. Obermeier *et al.* [50] evaluated the oral bioavailability of 5 ester prodrugs of BMS-183920. The prodrugs exhibited improved (5-15 fold increase) cellular permeability through the Caco-2 cell monolayer compared to BMS-183920. However, despite this improvement in flux, the maximum improvement in oral bioavailability was only 2-fold with the dioxolenone and pivaloyloxyisobutyl prodrugs. Minimal

improvement in oral bioavailability in the rat was observed with the ethyl, propanoyloxyethyl and propanoyloxyisobutyl prodrugs.

Further studies [50] have examined the reasons for the failure of these prodrugs to produce greater improvements in oral bioavailability. The simple ethyl ester compound had a 9-fold higher flux than BMS-183920 through Caco-2 cells and a 7-fold higher absorption in bile duct cannulated rats. However, this prodrug was inefficiently converted to BMS-183920 *in vivo*, with circulating concentrations of prodrug being observed up to 12 hours after dosing. Furthermore, the ethyl ester prodrug was also N-glucuronidated and subject to oxidative metabolism. Thus, the increased lipophilicity of the prodrug (relative to BMS-183920) increases either the access to or clearance by non-esterase drug metabolising enzymes. Indeed, for prodrugs of BMS-183920, glucuronidation of both the carboxylic acid and tetrazole functionalities was observed, whereas neither glucuronide conjugate was seen with BMS-183920.

A series of double esters were then synthesised, which had a range of hydrolytic stabilities. These showed a bell-shaped relationship between ester hydrolysis rate and oral bioavailability. If the hydrolysis was too slow, then non-productive metabolism occurred. If the hydrolysis rate was too high, oral bioavailability was also low (although the only circulating metabolite was BMS-183920), probably due to pre-systemic hydrolysis of the prodrug.

Overall, the many examples presented here highlight the potential pitfalls associated with a prodrug strategy. Improvement of oral absorption of a polar molecule by masking ionisable functions to increase lipophilicity is less than half the battle in designing a successful prodrug. Ensuring that the prodrug is processed in an appropriate manner post absorption, to quantitatively liberate the active principle is probably a more significant challenge. For these reasons, the prodrug approach should not be taken lightly. However, on some occasions, prodrugs may be the only option for oral delivery of important therapeutic agents. For these occasions, we now will discuss the most appropriate strategy for evaluating potential prodrug molecules in the Discovery setting.

THOUGHTS ON A DISCOVERY SCREENING STRATEGY

As stated several times, prodrug optimisation programs attempt to address two areas: transcellular oral absorption of the prodrug and release of the active principle. However, predicting prodrug disposition from *in vitro* data and *in vivo* animal experiments can be difficult as there are a large number of potential variables to consider. Also, many desirable prodrug properties can be in opposition. In addition, the understanding of esterase characteristics and disposition in various tissues is limited [94] and marked species differences in esterase distributions can limit the utility of some species, particularly rodents, for *in vivo* studies [95-98].

Profiling commercialised prodrugs with known clinical properties in order to understand the relationship between *in vitro*, *in vivo* and clinical findings may offset some of the issues detailed above. Fig. (11) represents an idealised

strategy for prodrug optimisation in discovery and the *in vitro* and *in vivo* assays that can be used in optimising prodrugs are discussed in more detailed below.

IN VITRO ASSAYS

In vitro assays allow the absorptive and metabolic properties of a prodrug to be rapidly evaluated. For example, *in vitro* experiments in human tissues allow rapid evaluation of a wide range of esters for their ability to release active principle. These *in vitro* systems may also provide valuable information on the species similarities/differences in productive and non-productive metabolism of prodrugs. The data obtained may be important in selecting species for *in vivo* examination of new chemical entities, as well as in interpreting the data generated.

ORAL ABSORPTION

Frequently, Caco-2 cell monolayers are used to evaluate the potential absorption/permeability of molecules [99]. This approach can be used for prodrugs. However, absorption of a prodrug can be modulated by metabolism/active transport in the enterocyte. Therefore, any methods of evaluating absorption should also consider ester hydrolysis rates and the fate of the active principle. Hydrolysis of prodrugs during passage across the Caco-2 cell has been observed along with active transport of the hydrolysed acid back into the apical compartment [77,98,100] and these data may have utility in guiding a prodrug program. However, due to the potential impact of metabolic and transport processes on the permeability assessment of prodrugs Caco-2 cells may not be representative of the human *in vivo* situation. Alternative approaches using human intestinal tissues could be considered, for example Ussing chamber experiments. These experiments can also be carried out with animal tissue to study the species differences in permeability, gut wall metabolism and efflux on the transit of prodrug across the intestine [76,101].

HYDROLYSIS OF THE PRODRUG

A wide range of *in vitro* systems exist to study the hydrolysis of prodrug esters. Pro-drug hydrolysis is most frequently characterised using a range of tissue fractions to determine the release of the active principle from the prodrug. These tissue fractions range from simple homogenates to microsomes [76,94,98,102] and are most frequently derived from the major first pass organs encountered following oral administration (liver, intestine).

Esterase hydrolysis can also occur in plasma/blood [96,97,103] and this site of hydrolysis can be particularly important as marked species differences in metabolic rate exist in this tissue, with rat frequently showing much higher rates of hydrolysis than man [95-97].

NON-PRODUCTIVE METABOLISM, BILIARY EXCRETION AND RELEASE FROM THE SITE OF HYDROLYSIS

The simple *in vitro* systems detailed above allow the evaluation of the rate of cleavage of the ester prodrug to the active principle, but do not permit examination of the balance of prodrug release and non-productive metabolism.

Fortifying these *in vitro* systems with the cofactors required for phase I and phase II metabolism may allow estimates of the relative balance between these metabolic processes [50,59]. Also canalicular membrane vesicles have been used to study the biliary transport of trandoloprilat and temocaprilat [78] and these techniques could be used to investigate non-productive clearance of prodrug or active principle by biliary excretion.

Suspension cultures of hepatocytes can be used for studying the metabolism and release of prodrug molecules. Hepatocytes maintain an intact cell membrane and also contain the esterases required to hydrolyse prodrug along with the other competing metabolic processes. Therefore hepatocytes may provide data on the hydrolysis rate of a variety of esters, the impact of competing metabolic processes and the release of ester from the hepatocyte. However, if an active transport process is responsible for non-productive clearance of the active principle or prodrug, hepatocytes may be of limited utility as these transporters cannot be readily evaluated (excretion at the biliary canalicular membrane leads to release of the active principle in to the incubation media) and to date there is no consistent evidence that active uptake studies can be performed in cryopreserved human hepatocytes.

In vitro experiments in human tissues allow rapid evaluation of a wide range of esterases for their ability to metabolise a prodrug to its active principle. These *in vitro* systems may also provide valuable information on the species similarities/differences in the productive and non-productive metabolism of prodrugs. The data obtained may be important in selecting species for *in vivo* examination of new chemical entities, as well as in interpreting the data generated. More complex *in vitro* experiments may provide information on both non-productive metabolism and release of prodrug.

IN VIVO ASSAYS FOR INVESTIGATING PRODRUG PROPERTIES

Marked species differences in esterase liability can make pharmacokinetic evaluations of esterase labile prodrugs difficult. For example, rodent species frequently have higher plasma esterase rates than human [95-97]. If this is the case, release of the active principle from sites of hydrolysis is less important in rodents than in man. Therefore, the pharmacokinetic data generated in rodents may represent an optimistic view of the active principle release in human [23].

These species differences in esterase metabolism make it important to select at least one species for pharmacokinetic evaluation that has a similar esterase profile to human.

Intravenous and oral pharmacokinetic studies with both the active principle and a range of prodrugs will provide information about the profile of the molecules [97]. For example, absolute oral bioavailability must be determined for prodrug and active principle. This is vital to ensure that the prodrug is providing a benefit. Prodrug oral bioavailability data obtained in both rat and dog can provide a useful guide to the likely oral bioavailability in human (Table 9). However, the inference from these data is that neither rat nor dog can be relied upon as a predictor for human oral bioavailability. Consequently, *in vivo* oral bioavailability determinations do not provide complete confidence of the likely outcome in human. Intravenous administration of the prodrug could be used to confirm that the molecule is hydrolysed and released *in vivo*. Whilst compound administered by the intravenous route will not undergo the first-pass metabolism seen following oral administration these studies may allow ranking of compounds for further investigation. These data coupled with the relevant *in vitro* data should allow an estimate of the likely absorption of a prodrug and release of the active principle in humans. However, because of the complexity of the process involved in prodrug release, it is unlikely that comprehensive *in vitro* and *in vivo* experimental packages will provide complete confidence in the pharmacokinetic profile of the prodrug in human [94]. In light of this it may be appropriate to consider evaluating more than one prodrug in humans and making a final selection using human pharmacokinetic data.

CONCLUSIONS

The aim of this review has been to highlight the difficulties around the prodrug approach to improving the oral absorption of polar molecules. Clearly, prodrug strategies have been successful for a number of important therapeutic agents. However, further investigation suggests that the hurdles to oral delivery of an ester prodrug are not trivial. These include maintaining sufficient aqueous solubility, lipophilicity and chemical stability at the same time as enabling rapid and quantitative release of active principle post absorption. In addition, significant non-esterase metabolism and transporter mediated clearance of the prodrug is not desirable. For these reasons, it appears that

Table 9. Bioavailability Values of Active Principles Following Oral Dosing of Prodrugs

Prodrugs	Rat	Dog	Human
Candesartan Cilexetil [134]	27.8	5	42
Quinapril [135]	63	47	46-52
Enalapril* [20]	34	61	36-44
Tenofovir [62]	ND	30	27-41 (> with food)
Candoxatril [23]	53	17	32

* absorption

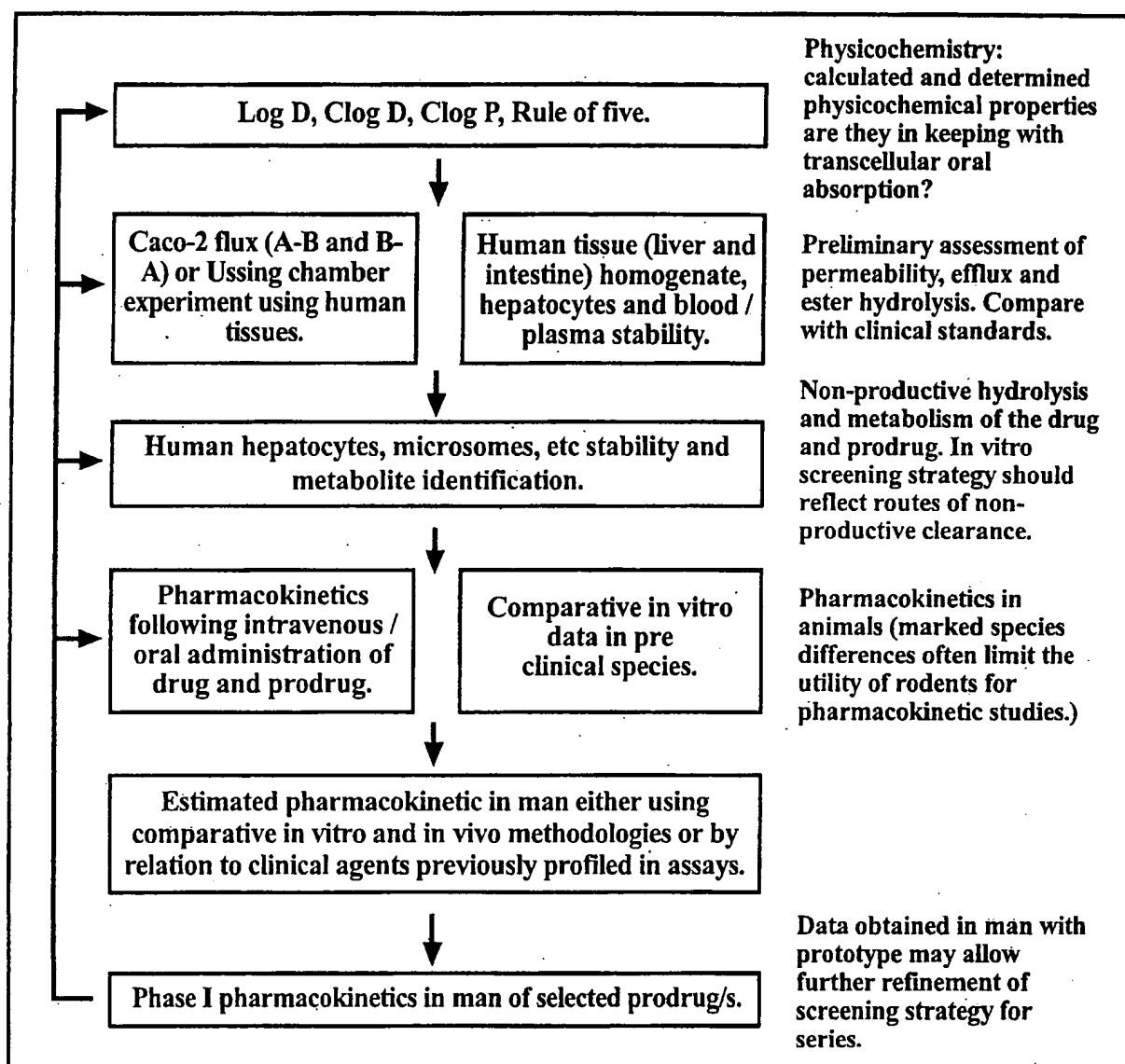


Fig. (11). Proposed screening strategy for a prodrug program.

achieving high oral bioavailability values with a prodrug approach is extremely difficult and a realistic target for oral bioavailability would be 50%. In addition, designing an ester prodrug that balances all of these issues is a difficult undertaking and a robust screening sequence is required in the Discovery environment. However, due to the difficulty in predicting the human disposition of an ester prodrug, it may be necessary to evaluate several examples in human pharmacokinetic studies. Given the complexities outlined in this review, it is recommended that the prodrug strategy is only considered as a last resort to improve the oral bioavailability of important therapeutic agents.

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